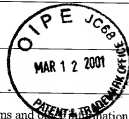


U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER MOROZ3
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/786867
INTERNATIONAL APPLICATION NO. PCT/IL99/00485	INTERNATIONAL FILING DATE 8 September 1999	PRIORITY CLAIMED 11 September 1998
TITLE OF INVENTION DNA SEQUENCE ENCODING ONCOFETAL FERRITIN PROTEIN		
APPLICANT(S) FOR DO/EO/US C. MOROZ		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and information:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). <input checked="" type="checkbox"/> The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31). <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is attached hereto (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been communicated by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been communicated by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern document(s) or information included:</p> <ol style="list-style-type: none"> <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. <input type="checkbox"/> An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input checked="" type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> Other items or information: <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Courtesy copy of the International Application as filed. <input checked="" type="checkbox"/> Courtesy copy of the first page of the International Publication (WO 00/15788). <input checked="" type="checkbox"/> <u>Courtesy copy of the International Preliminary Examination Report with annexes containing claims 1-28 to be substituted for original claims 1-29 for examination in this case.</u> <input checked="" type="checkbox"/> Formal drawings, 15 sheets, Figures 1-12. <input checked="" type="checkbox"/> Courtesy Copy of the International Search Report. 		



U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <div style="font-size: 24pt; font-weight: bold; text-align: center;">09/786867</div>	International Application No PCT/IL99/00485	Attorney's Docket No MOROZ3
---	---	---------------------------------------

17. [xx] The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00	<div style="border: 1px solid black; padding: 5px;">CALCULATIONS PTO USE ONLY</div>
--	--

ENTER APPROPRIATE BASIC FEE AMOUNT =	\$ 860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$ 130.00	

Claims as Originally Presented	Number Filed	Number Extra	Rate		
Total Claims	22 - 20	2	X \$18.00	\$ 36.00	
Independent Claims	5 - 3	2	X \$80.00	\$ 160.00	
Multiple Dependent Claims (if applicable)			+ \$270.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 1,186.00	

Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate		
Total Claims	38 - 22	16	X \$18.00	\$ 288.00	
Independent Claims	5 - 5	0	X \$80.00	\$ 0	
TOTAL OF ABOVE CALCULATIONS =				\$ 1,474.00	

Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.

SUBTOTAL =	\$ 1,474.00	
Processing fee of \$130.00 for furnishing the English translation later than [] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	\$	
TOTAL NATIONAL FEE =	\$1,474.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +	\$	
TOTAL FEES ENCLOSED =	\$1,474.00	

	Amount to be:
	refunded
	charged

a. [] A check in the amount of \$_____ to cover the above fees is enclosed.
b. [xx] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of \$ 1,474.00, is attached.
c. [] Please charge my Deposit Account No. **02-4035** in the amount of \$_____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
d. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive 37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
624 NINTH STREET, N.W., SUITE 300
WASHINGTON, D.C. 20001
TEL: (202) 628-5197
FAX: (202) 737-3528
Date of this submission: March 12, 2001

SIGNATURE
Roger L. Browdy
 NAME
25,618
 REGISTRATION NUMBER

Rec'd PCT/PTO 21 AUG 2001

#6/C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Box PCT
MOROZ, Chaya)	Examiner:
Appln. No.: 09/786,867)	Washington, D.C.
IA No. PCT/IL99/00485)	
IA Filed: September 8, 1999)	August 21, 2001
For: DNA SEQUENCE ENCODING)	Atty.Docket: MOROZ=3
ONCOFETAL FERRITIN PROTEIN))	

RESPONSE TO NOTIFICATION TO COMPLY WITH
SEQUENCE LISTING REQUIREMENTS

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification to Comply dated May 4, 2001, a petition for a two-month extension of time being attached hereto, and prior to the examination of the above-described application, please amend the present application as follows:

IN THE SPECIFICATION

Please replace the table on page 15 with the following rewritten table:

09786867-082101

5' GGT GGC GAC GAC TCC TGG AGC CCG 3'	SEQ ID NO:6
5' TTG ACA CCA GAC CAA CTG GTA ATG 3'	SEQ ID NO:7
5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'	SEQ ID NO:8
5' GAT AGG ATC TTT AGC GAC AGC CGA 3'	SEQ ID NO:9
5' ATG GCG GCC TCT GAG TCC TGG TGG 3'	SEQ ID NO:10
5' CGG GCT GAA TGC AAT GGA GTG TGC 3'	SEQ ID NO:11
5' GAC CCC CAT TTG TGT GAC 3'	SEQ ID NO:12
5' CGA CGA CTC CTG GAG CCC G 3'	SEQ ID NO:13
5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'	SEQ ID NO:14
5' AGC CGA CAG CGA TTT CTA GGA TAG 3'	SEQ ID NO:15
5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'	SEQ ID NO:16
5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'	SEQ ID NO:17
5' CAG ACG TTC TTC GCC GAG AGT CGT 3'	SEQ ID NO:18
5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'	SEQ ID NO:19
5' CAT TTC GGG GAT TCG GGG GA 3'	SEQ ID NO:20
5' GGG GGA CGG AAC CCG GCG CT 3'	SEQ ID NO:21
5' CCC TCT ACA CTT ATC ATC TTC 3'	SEQ ID NO:22
5' CTA TCC TAG AAA TCG CTG TCG GCT 3'	SEQ ID NO:23
5' GTC ACT ACT GGA ATT CCC TTC TCC 3'	SEQ ID NO:24
5' GGA GAA GGG AAT TCC AGT AGT GAC 3'	SEQ ID NO:25
5' GGA AAT CGC TGT CGC CTA ACC 3'	SEQ ID NO:26
5' GGT TAG GCG ACA GCG ATT TCC 3'	SEQ ID NO:27
5' GGC CAC GCG TCG ACT AGT AC 3'	SEQ ID NO:28
5' GTA ATG CAC ACTCCA TTG GC 3'	SEQ ID NO:29
5' GTA ATG CAC ACT CCA TTG 3'	SEQ ID NO:30
5' GCG CTC AGC TGG AAT TCC 3'	SEQ ID NO:31
5' GGA ATT CCA GCT GAG CGC 3'	SEQ ID NO:32
5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3'	SEQ ID NO:33
5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'	SEQ ID NO:34
5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'	SEQ ID NO:35
5' CCC GCT CGA GTC AGG GTG ACC GAA AAA TCA G 3'	SEQ ID NO:36

Please replace the two paragraphs beginning at line 6 on page 16 and ending at line 13 of page 16 with the following rewritten paragraphs:

--Fig. 1 shows the nucleic acid sequence (SEQ ID NO:1) of clone T16 isolated from T47D breast cancer cDNA library. Initiation and termination codons of the open reading frame are indicated by dark bars;

Fig. 2A shows a comparison of the nucleic acid sequences (upper sequence) (SEQ ID NO:2) of clone 4.7 isolated from a placenta cDNA library exhibiting normal human FTH, and the sequences (lower sequence) of clone T16 (SEQ ID NO:1) isolated from human breast cancer T47D cDNA library. Initiation and termination codons of the open reading frame are marked by dark boxes;--

Please replace the three paragraphs beginning at line 17 of page 16 and ending at line 25 of page 16 with the following rewritten paragraphs:

--Fig. 3 shows a comparison of sequence homology between cDNA clone T16 (residues 463-671 of SEQ ID NO:1) and human mitochondrial cytochrome oxidase I DNA (SEQ ID NO:3);

Fig. 4 shows a comparison of nucleic acid sequences between placental cDNA obtained by PCR amplification using T16 specific primers (upper sequence) (residues 24-822 of SEQ ID NO:1) and T16 cDNA sequence obtained from the T16 cDNA clone (lower sequence) (SEQ ID NO:4). Identical nucleic acid sequences are indicated by a dotted line. Initiation and termination codons are indicated by a dark bar;

Fig. 5 shows the nucleic acid sequence and deduced amino acid sequence (SEQ ID NO:5) of the cDNA of OFF1;--

Please replace the two paragraphs beginning at line 4 of page 17 and ending at line 6 of page 17 with the following rewritten paragraphs:

--**Fig. 7** shows the sequence of clone T16 (SEQ ID NO:1).

Primers used for PCR are indicated in the above sequence;

Fig. 8 shows the restriction enzyme map sequence of clone T16 (SEQ ID NO:1);--

09786867-002101

Please replace Table 1 on page 19 with the following
rewritten Table 1:

Table 1
List of Primers

Name	#MR	Sequence	SEQ ID NO:		
1060F	24	5' GGT GGC GAC GAC TCC TGG AGC CCG 3'	6	75%	
1061R	24	5' TTG ACA CCA GAC CAA CTG GTA ATG 3'	7	45.80%	
17F	27	5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'	8	52%	27618
X1.1F	24	5' ATG AGG ATC TTT AGC GAC AGC CGA 3'	9	50%	24880
X1.1R	24	5' ATG GCG GCC TCT GAG TCC TGG TGG 3'	10	67%	
2.1F	24	5' CGG GCT GAA TGC AAT GGA GTG TGC 3'	11	58%	
3.4F	18	5' GAC CCC CAT TTG TGT GAC 3'	12	55.50%	
1060F/S	19	5' CGA CGA CTC CTG GAG CCC G 3'	13	73.70%	
1061r/Bio	24	5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'	14	45.80%	
16X.1R	24	5' AGC CGA CAG CGA TTT CTA GGA TAG 3'	15	50%	24879
17R	27	5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'	16	52%	27385
3'COD R	28	5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'	17	50%	28313
5' NCF	24	5' CAG ACG TTC TTC GCC GAG AGT CGT 3'	18	58%	24870
4869	27	5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'	19	63%	
NFG	20	5' CAT TTC GGG GAT TCG GGG GA 3'	20	60%	
NFGP-2	20	5' GGG GGA CGG AAC CCG GCG CT 3'	21	80%	201880
767-F	21	5' CCC TCT ACA CTT ATC ATC TTC 3'	22	43%	211616
16-F	24	5' CTA TCC TAG AAA TCG CTG TCG GCT 3'	23	50%	241173
ECO-F	24	5' GTC ACT ACT GGA ATT CCC TTC TCC 3'	24	50%	24960
ECO-R	24	5' GGA GAA GGG AAT TCC AGT AGT GAC 3'	25	50%	24961
SPF	21	5' GGA AAT CGC TGT CGC CTA ACC 3'	26	57%	211667
SPR	21	5' GGT TAG GCG ACA GCG ATT TCC 3'	27	57%	211668
AUAP	20	5' GGC CAC GCG TCG ACT AGT AC 3'	28	65%	202738
NC-F	20	5' GTA ATG CAC ACTCCA TTG GC 3'	29	50%	203814
SNC-F	18	5' GTA ATG CAC ACT CCA TTG 3'	30	44%	181897
BNC-F	18	5' GCG CTC AGC TGG AAT TCC 3'	31	55.50%	181898
BNC-R	18	5' GGA ATT CCA GCT GAG CGC 3'	32	61.10%	181905
pGEX-F	29	5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC3'	33	67%	29391
pGEX-R1	27	5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'	34	51.85%	27578
pGEX-R2	29	5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'	35	51.70%	29396
pGEX-R3	31	5' CCCGCTCGAGTCAGGGTGACCGAAAAATCAG 3'	36	58%	31277

Please replace the paragraph beginning at line 6 on page 27 with the following rewritten paragraph:

--The expression vector (pGEX-5X-1) used for gene fusion construction was the GST Gene Fusion System (Pharmacia). The OFF1 coding region (designated as "FL", full-length) of about 0.5 kb was prepared by PCR with the following 5' end primer:

5' GTGGGATCCCCATGACGACCGCGTCCA (1-27 of SEQ ID NO:33), in order
BamHI

to add a BamHI site 1 base upstream from the start codon ATG and with the 3' end primer

5' CCCG CTCGAG TCA GGG TGA CCG AAA AAT CAG 3' (SEQ ID NO:36) in
XhoI

order to add an XhoI site after the stop codon TAA using the PCR kit (Perkin-Elmer/Cetus).--

IN THE DRAWINGS

Attached hereto are copies of Figures 1, 2A, 5,, 7 and 8 with proposed revisions marked in red. Approval of these revisions is respectfully requested.

IN THE SEQUENCE LISTING

Please substitute the attached Sequence Listing, numbered as pages 1-8 for the Sequence Listing previously submitted.

REMARKS

Applicants have substituted into the present specification a new paper copy Sequence Listing section according to 37 C.F.R. §1.821(c) as new pages 1-8.

Applicants have amended the previously filed Sequence Listing and revised Figures 1, 2A, 5, 7 and 8 to make them conform to the amended Sequence Listing.

SEQ ID NO:1 of the previously filed Sequence Listing corresponded to originally filed Figure 1. Originally filed Figure 1 erroneously duplicated the lower nucleotide sequence presented in Figure 4 (which lower sequence now corresponds to SEQ ID NO:4 in the attached substitute Sequence Listing). SEQ ID NO:1 of the Sequence Listing and Figure 1 have been amended to conform to the nucleotide sequence shown Figs. 2A (lower sequence), 5, 7 and 8. These amendments are supported by the disclosure in the specification of Figs. 1, 2A, 5, 7 and 8, that SEQ ID NO:1 is identical in all these figures.

The amendment to SEQ ID NO:2 was necessitated due to an erroneous addition of incorrect nucleotides at positions 886-953. These incorrect nucleotides have now been deleted from SEQ ID NO:2 and this amendment is supported by the nucleotide sequence shown in Figure 2A (upper sequence).

SEQ ID NO:3 has been deleted from the previously filed Sequence Listing as SEQ ID NO:3 was a partial sequence of SEQ ID NO:1. The subsequent sequences have been renumbered accordingly in the attached substitute Sequence Listing.

The revisions to Figs. 1, 2A and 5 were necessitated due to typographical/clerical errors made when drafting these figures, and were made to make the sequences of Figs. 1, 2A and 5 conform to SEQ ID NO:1.

The revisions to Figures 7 and 8 merely correct an alignment error in the second column of the last row of the sequence.

All of the above revisions to the Sequence Listing and the figures are of clerical/typographical nature and do not constitute new matter. Approval of all of the revisions is therefore respectfully requested.

Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

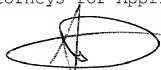
Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By



ALLEN C. YUN

Registration No. 37,971

ACY:al
624 Ninth Street, N.W.
Washington, D.C. 20001
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Facsimile No.: (202) 737-3528

F:\c\cohn\morez3\pto\RESPONSE TO NOTICE TO COMPLY.doc

VERSION WITH MARKINGS TO SHOW CHANGES MADEIn the specification:

The table on page 15 has been amended as follows:

5' GGT GGC GAC GAC TCC TGG AGC CCG 3'	SEQ ID NO:6
5' TTG ACA CCA GAC CAA CTG GTA ATG 3'	SEQ ID NO:7
5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'	SEQ ID NO:8
5' GAT AGG ATC TTT AGC GAC AGC CGA 3'	SEQ ID NO:9
5' ATG GCG GCC TCT GAG TCC TGG TGG 3'	SEQ ID NO:10
5' CGG GCT GAA TGC AAT GGA GTG TGC 3'	SEQ ID NO:11
5' GAC CCC CAT TTG TGT GAC 3'	SEQ ID NO:12
5' CGA CGA CTC CTG GAG CCC G 3'	SEQ ID NO:13
5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'	SEQ ID NO:14
5' AGC CGA CAG CGA TTT CTA GGA TAG 3'	SEQ ID NO:15
5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'	SEQ ID NO:16
5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'	SEQ ID NO:17
5' CAG ACG TTC TTC GCC GAG AGT CGT 3'	SEQ ID NO:18
5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'	SEQ ID NO:19
5' CAT TTC GGG GAT TCG GGG GA 3'	SEQ ID NO:20
5' GGG GGA CGG AAC CCG GCG CT 3'	SEQ ID NO:21
5' CCC TCT ACA CTT ATC ATC TTC 3'	SEQ ID NO:22
5' CTA TCC TAG AAA TCG CTG TCG GCT 3'	SEQ ID NO:23
5' GTC ACT ACT GGA ATT CCC TTC TCC 3'	SEQ ID NO:24
5' GGA GAA GGG AAT TCC AGT AGT GAC 3'	SEQ ID NO:25
5' GGA AAT CGC TGT CGC CTA ACC 3'	SEQ ID NO:26
5' GGT TAG GCG ACA GCG ATT TCC 3'	SEQ ID NO:27
5' GGC CAC GCG TCG ACT AGT AC 3'	SEQ ID NO:28
5' GTA ATG CAC ACTCCA TTG GC 3'	SEQ ID NO:29
5' GTA ATG CAC ACT CCA TTG 3'	SEQ ID NO:30
5' GCG CTC AGC TGG AAT TCC 3'	SEQ ID NO:31
5' GGA ATT CCA GCT GAG CGC 3'	SEQ ID NO:32
5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3'	SEQ ID NO:33
5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'	SEQ ID NO:34
5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'	SEQ ID NO:35
5' CCC GCT CGA GTC AGG GTG ACC GAA AAA TCA G 3'	SEQ ID NO:36

The two paragraphs beginning at line 6 on page 16 and ending at line 13 of page 16 have been amended as follows:

Fig. 1 shows the nucleic acid sequence (SEQ ID NO:1) of clone T16 isolated from T47D breast cancer cDNA library. Initiation and termination codons of the open reading frame are indicated by dark bars;

Fig. 2A shows a comparison of the nucleic acid sequences (upper sequence) (SEQ ID NO:2) of clone 4.7 isolated from a placenta cDNA library exhibiting normal human FTH, and the sequences (lower sequence) of clone T16 (SEQ ID NO:1) isolated from human breast cancer T47D cDNA library. Initiation and termination codons of the open reading frame are marked by dark boxes;

The three paragraphs beginning at line 17 of page 16 and ending at line 25 of page 16 have been amended as follows:

Fig. 3 shows a comparison of sequence homology between cDNA clone T16 (residues 463-671 of SEQ ID NO:1) and human mitochondrial cytochrome oxidase I DNA (SEQ ID NO:3);

Fig. 4 shows a comparison of nucleic acid sequences between placental cDNA obtained by PCR amplification using T16 specific primers (upper sequence) (residues 24-822 of SEQ ID NO:1) and T16 cDNA sequence obtained from the T16 cDNA clone (lower sequence) (SEQ ID NO:4). Identical nucleic acid sequences are indicated by a dotted line. Initiation and termination codons are indicated by a dark bar;

Fig. 5 shows the nucleic acid sequence and deduced amino acid sequence (SEQ ID NO:5) of the cDNA of OFF1;

The two paragraphs beginning at line 4 of page 17 and ending at line 6 of page 17 have been amended as follows:

Fig. 7 shows the sequence of clone T16 (SEQ ID NO:1).
pPrimers used for PCR are indicated in the above sequence;

Fig. 8 shows the restriction enzyme map sequence of clone T16 (SEQ ID NO:1);

Table 1 on page 19 has been amended as follows:

Table 1
List of Primers

Name	#MR	Sequence	SEQ_ID NO:		
1060F	24	5' GGT GGC GAC GAC TCC TGG AGC CCG 3'	6	75%	
1061R	24	5' TTG ACA CCA GAC CAA CTG GTA ATG 3'	7	45.80%	
17F	27	5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'	8	52%	27618
X1.1F	24	5' GAT AGG ATC TTT AGC GAC AGC CGA 3'	9	50%	24880
X.1.1R	24	5' ATG GCG GCC TCT GAG TCC TGG TGG 3'	10	67%	
2.1F	24	5' CGG GCT GAA TGC AAT GGA GTG TGC 3'	11	58%	
3.4F	18	5' GAC CCC CAT TTG TGT GAC 3'	12	55.50%	
1060F/S	19	5' CGA CGA CTC CTG GAG CCC G 3'	13	73.70%	
1061r/Bio	24	5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'	14	45.80%	
16X.1R	24	5' AGC CGA CAG CGA TTT CTA GGA TAG 3'	15	50%	24879
17R	27	5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'	16	52%	27385
3'COD R	28	5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'	17	50%	28313
5' NCF	24	5' CAG ACG TTC TTC GCC GAG AGT CGT 3'	18	58%	24870
4869	27	5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'	19	63%	
NFG	20	5' CAT TTC GGG GAT TCG GGG GA 3'	20	60%	
NFGP-2	20	5' GGG GGA CGG AAC CCG GCG CT 3'	21	80%	201880
767-F	21	5' CCC TCT ACA CTT ATC ATC TTC 3'	22	43%	211616
16-F	24	5' CTA TCC TAG AAA TCG CTG TCG GCT 3'	23	50%	241173
ECO-F	24	5' GTC ACT ACT GGA ATT CCC TTC TCC 3'	24	50%	24960
ECO-R	24	5' GGA GAA GGG AAT TCC AGT AGT GAC 3'	25	50%	24961
SPF	21	5' GGA AAT CGC TGT CGC CTA ACC 3'	26	57%	211667
SPR	21	5' GGT TAG GCG ACA GCG ATT TCC 3'	27	57%	211668
AUAP	20	5' GGC CAC GCG TCG ACT AGT AC 3'	28	65%	202738
NC-F	20	5' GTA ATG CAC ACTCCA TTG GC 3'	29	50%	203814
SNC-F	18	5' GTA ATG CAC ACT CCA TTG 3'	30	44%	181897
BNC-F	18	5' GCG CTC AGC TGG AAT TCC 3'	31	55.50%	181898
BNC-R	18	5' GGA ATT CCA GCT GAG CGC 3'	32	61.10%	181905
pGEX-F	29	5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC3'	33	67%	29391
pGEX-R1	27	5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'	34	51.85%	27578
pGEX-R2	29	5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'	35	51.70%	29396
pGEX-R3	31	5' CCCGCTCGAGTCAGGGTGACCGAAAAATCAG 3'	36	58%	31277

The paragraph beginning at line 6 on page 27 has been amended as follows:

The expression vector (pGEX-5X-1) used for gene fusion construction was the GST Gene Fusion System (Pharmacia). The OFF1 coding region (designated as "FL", full-length) of about 0.5 kb was prepared by PCR with the following 5' end primer:

5' GTGGGATCCCCATGACGACGCGTCCA (1-27 of SEQ ID NO:33), in order
BamHI

to add a BamHI site 1 base upstream from the start codon ATG and with the 3' end primer

5' CCCG CTCGAG TCA GGG TGA CCG AAA AAT CAG 3' (SEQ ID NO:36) in
XhoI

order to add an XhoI site after the stop codon TAA using the PCR kit (Perkin-Elmer/Cetus).

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5' TTGACACCAG ACCAACTGGT AATGGTAGCC ACCGGCGCTC AGCTGG^AATT ^{AA}CC~~T~~AAAATG
 TAATGCACAC TCCATTG~~A~~CAT TCAGCCCGCC TCCTCTTAGT CGCCGCC~~ATC~~ ACGACCGCGT
 CCACCTCGCA GGTGCGCCAG AACTACCACC AGGACTCAGA GGGCGCCATC AACCGCCAGA
 TCAACCTGGA GCTCTACGCC TCCTACGTTT ACCTGTCCAT GTCTTACTAC TTTGACCGCG
 ATGATGTGGC TTTGAAGAAC TTTGCCAAAT ACTTTCTTCA CCAATCTCAT GAGGAGAGGG
 A~~A~~CATGCTGA GAAAGTGATG AAGCTGCAGA ACCAACGAGG TGGCCGAATC TTCCTTCAGG
 ATATCAAGAA ACCGACTGTG GATGACTGGG AGAGCGGGCT GAATCCAATG GAGTGTGCAT
 TACATTGGGA AAAAAATGTG AATCAGTCAC TACTGGAATT CCCTTCTCCT ATCTCTCCCA
 GTCTAGCTG CTGGCATCAC TATACTACTA ACAGACCGCA ACCTCAACAC CACCTTCTTC
 GACCCCGCCG GAGGAAGAGA CCCCATTCTA TACCAACACC TATTCTGATT TTTGGTCCAC
 CCTGA~~A~~AGTTT ATATTCTTAT CCTACCAGGC TTCGGAATAA TCTCCCATAT TGTAACTTAC
 TACTCGGGAA ATCGCTGTG CCTAACCGCT AACATTACTG CAGGCCACCT ACTCATGCAC
 CTAATTGGAA GCGCCACCCT AGCAATATCA ACCAATTAACC TTCCTCTAC ACTTATCATC
 TTCACAATC TAATTCTACT GACTATCCTA GAAATCGGTG TCGCCTTAAT CCAAGCCTAC
 GTTTTCACAC TTCTAGTAA~~G~~ ~~A~~CCTCTACCT ~~A~~ACAGACAAC~~A~~ ~~A~~CATAAAAAA~~A~~ ~~A~~ 3'

Fig. 1

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Fig. 2A

CLONE p47GGGGGACGGAA⁶CCGG

CGCTCGTTCCCCACCCGGCCGGCCGCCATAGCCAGCCCTCCGTAC

CLONE T 16TTGACACCG~~7~~

CTCTTCACCGCACCCCTCGGACTGCCCAAGGCCCGCCGCCGTCC

A⁸ACCAACTGGTAATGGTAGCGACCGCGCTCAGCTGGAATTC¹⁰AAAA

AGCGCCGCGCAGCCACCGCGCCGCCGCCGCTCTCCTTAGTCGCCGCGC

AATGTAATGCACACTCCATTGCATTGACCCCGCCTCTCCTTAGTCGCCGCGC

ATG	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG
ATG	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG

AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC

CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC

GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC

GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC

TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT
TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT

GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA

GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA

CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT

GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT

GTG	AAT	CAG	TCA	CTA	CTG	GAA	CTG	CAC	AAA	CTG
GTG	AAT	CAG	TCA	CTA	CTG	GAA	TTC	CCT	TCT	CCT

GCC	ACT	GAC	AAA	AAT	GAC	CCC	CAT	TTG	TGT	GAC
ATC	TCT	CCC	AGT	CCT	AGC	TGC	TGG	CAT	CAC	TAT

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TTC ATT GAG ACA CAT TAC CTG AAT GAG CAG GTG
 ACT ACT AAC AGA CCG CAA CCT CAA CAC CAC CTT

AAA GCC ATC AAA GAA TTG GGT GAC CAC GTG ACC
 CTT CGA CCC CGC CGG AGG AAG AGA CCC CAT TCT

AAC TTG CGC AAG ATG GGA GCG CCC GAA TCT GGC
 ATA CCA ACA CCT ATT CTG ATT TTT CGG TCA CCC

TTG GCG GAA TAT CTC TTT GAC AAG CAC ACC CTG
 TGA AGTTTATATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATT

GGA GAC AGT GAT AAT GAA AGC TAA GCCTCGGGCTAATT
 GTAACCTTACTACTCCGGAATCGCTGTCGCCTAACCGCTAACATTACTGC

TCCCATAGCCGTGGGGTGACTTCCTGGTCACCAAGGCAGTGCAATGCAT
 AGGCCACCTACTCATGCACCTAATTGGAAGCGCCACCTAGCAATATCA

GCATGTTGGGGTTTCCTTTACCTTTTCTATAAGTTGTACCAAAACATCCAC
 ACCATTAACCTTCCCTCTACACTTATCATCTTACAAATTCTAATTCTACTG

TTAAGTCTTTGATTTGTACCATTCCTTCAAATAAGAAATTTGGTACCCA
 ACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTACGTTTTACACT

AAAAAAA
 C
 TCTAGTAAGCCTCTACCTGCACGACAAACATAAAAAA

Fig. 2A Cont.

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TTGACACCAGACCAACTGGTAATGGTAGCGACCGCGGCTCAGCTGGAATTCCAAAAATGT

AATGCACACTCCATTGCATTGACCCGCGCTCTCCTTAGTCGCGCGC

met	thr	thr	ala	ser	thr	ser	gln	val	arg	gln
ATG	ACG	ACC	GCG	TCC	ACC	TGG	CAG	GTG	CGC	CAG
asn	tyr	his	gln	asp	ser	glu	ala	ala	ile	asn
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC
arg	gln	ile	asn	leu	glu	leu	tyr	ala	ser	tyr
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC
val	tyr	leu	ser	met	ser	tyr	tyr	phe	asp	arg
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC
asp	asp	val	ala	leu	lys	asn	phe	ala	lys	tyr
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC
phe	leu	his	gln	ser	his	glu	glu	arg	gln	his
TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT
ala	glu	lys	leu	met	lys	leu	gln	asn	gln	arg
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA
gly	gly	arg	ile	phe	leu	gln	asp	ile	lys	lys
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA
pro	asp	cys	asp	asp	trp	glu	ser	gly	leu	asn
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT
ala	met	glu	cys	ala	leu	his	leu	glu	lys	asn
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT
val	asn	gln	ser	leu	leu	glu	phe	pro	ser	pro
GTG	AAT	CAG	TCA	CTA	CTG	GAA	TTC	CCT	TCT	CCT
ile	ser	pro	ser	pro	ser	cys	trp	his	his	thr
ATC	TCT	CCC	AGT	CCT	AGC	TGC	TGG	CAT	CAC	TAT
thr	thr	asn	arg	pro	glu	pro	gln	his	his	leu
ACT	ACT	AAC	AGA	CCG	CAA	CCT	CAA	CAC	CAC	CTT
leu	arg	pro	arg	arg	arg	lys	arg	pro	his	ser
CTT	CGA	CCC	CGC	CGG	AGG	AAG	AGA	CCC	CAT	TCT
ile	pro	thr	pro	ile	leu	ile	phe	arg	ser	pro
ATA	CCA	ACA	CCT	ATT	CTG	ATT	TTT	CGG	TCA	CCC

TGA AGTTTATATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACCTAC

TACTCCGGAATCGCTGTCGCCTAACCGCTAACATTACTGCAGGCCACCTACTCATGCAC

CTAATTGGAAGCGCCACCCCTAGCAATATCAACCATTAAACCTTCCTCTACACTTATCATC

TTCACAATTCTAATTCTACTGACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTAC

GTTTTCACACTT

CTAGTAA GCCTCTACTCTGCACGACAA CACTAAAAAAA

Fig. 5

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<u>1061</u> T GACACCAG	<u>ACCAACTGGT</u>	<u>AATGGTAGCG</u>	<u>BNC</u> ACCGCGGCTC	<u>AGCTGGAAT</u>	<u>CCAAAAAATG</u>
<u>NCS</u> <u>TAATGCACAG</u>	<u>TCCATTGCAT</u>	TCAGCCCGCC	TCTCCTTAGT	CGGCCCGCATG	ACGACCGCGT
CCACCTCCGA	GGTGCGCCAG	AACTACCACC	<u>XI</u> <u>AGGACTCAGN</u>	<u>GCGCCGCATC</u>	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCTACGTTT	ACCTGTCCAT	GTCTTACTAC	<u>17</u> TTT <u>AGCCGCG</u>
<u>17</u> <u>ATGATGTGGC</u>	<u>TTTGAAGAAC</u>	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAG <u>CGGGCT</u>	<u>21</u> <u>GAATGCAATC</u>	<u>GAGTGTGCAT</u>
TACATTGGA	AAAAAATGTG	AATCA <u>GTCA</u>	<u>ECOF</u> <u>TACTGGAAT</u>	<u>CCCTTCTCGT</u>	ATCTCTCCCA
GTCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC
GACCCCGCGG	GAGGAAGAGA	COCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CCTGAAAGTTT	ATATTCTTAT	CCTACCAGGC	TTCGGAATAA	TCTCCATAT	TGTAACCTAC
TACTCC <u>GGA</u>	<u>SPF</u> <u>ATCGCTGTGG</u>	<u>CCTAACCGCT</u>	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATT <u>GAA</u>	<u>723</u> <u>GCGCCACCTT</u>	<u>AGCAATATCA</u>	<u>ACCAITTAACC</u>	TTCCCTC <u>TAC</u>	<u>767</u> <u>ACTTATCATC</u>
<u>767</u> <u>TCACAAATC</u>	<u>TAATTCTACT</u>	GACTATCCTA	<u>16</u> <u>GAAATCGCTG</u>	<u>TCGCGTTAAT</u>	CCAAGCCTAC
GTITTCACAC	TTCTAGTAAG	G CCTCTACCTG	G CACGACAACA	A CATAAAAAA	A

Fig. 7

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TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGCGCGTC	ACGTGGAATT	CCAAAAAATG
TAATGCACAC	TCCATTGCAT	TCAGCCTCGCC	TCCTCTAGT	CGCCGCCAAT	ACGACCGCGT
CCACCTCGCA	GCTGCGCCAG	AATACCACC	AGGACTGAGA	CGCCGCCATC	AACGCCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG
AACATCTGTA	GAAACTGATG	AAGCTGCAGG	ACCAACGAGG	TGGCCGAATC	TTCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT
TACATTGGA	AAAAAATGTG	AATCAGTCAC	TACTGGGAGT	CCCTTCTCCT	ATCTCTCCCA
GTCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTCTTTC
GACCCCGCGG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TAITCTGATT	TTTCGGTCAC
CCCTAGTTT	ATAITCTTAT	CCTACCAGGC	TTGGGAATAA	TCTCCCATAT	TGTAACTTAC
TACTCCGAA	ATCGTGTGCG	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	GCGCCACCGT	AGCAATATCA	ACCATTAAAC	TTCCCTCTAC	ACTTATCATC
TTCACAAITC	TAAITCTACT	GACTATCTTA	GAAATCGCTG	TGGCTTAAT	CCAAGCCTAC
GTITTCACAC	TTCTAGTAA	CCCTCTACCTG	CCACGACAACA	CCATAAAAAA	AA

Fig. 8

#5/
B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:) Art Unit:
MOROZ, C.)
)
IA No.: PCT/IL99/00485)
) Washington, D.C.
IA Filed: 8 September 1999)
)
U.S. App. No.:)
(Not Yet Assigned))
) March 12, 2001
National Filing Date:)
(Not Yet Received))
)
For: DNA SEQUENCE ENCODING...) Docket No.: MOROZ3

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please cancel claims 1-9, 11, 14-18, 20, 22-26 and 28 without prejudice in favor of the following new claims 29-66:

29. (New) A DNA sequence coding for oncofetal ferritin 1 (OFF1) protein selected from the group consisting of:

- (i) a DNA sequence as depicted in Fig. 1;
- (ii) a DNA sequence as depicted in Fig. 4;
- (iii) a DNA sequence which codes for the same amino acid sequences of (i) or (ii);
- (iv) fragments of any of the sequences of (i) to (iii) that code for a physiologically active protein;

(v) a DNA sequence that has at least 80% homology, as determined by hybridization under stringent conditions, to any one of the sequences of (i) to (iv) and code for a physiologically active protein; and

(vi) a DNA sequence that hybridizes to the sequences of (i) or (iv), under highly stringent conditions, being hybridization to filter-bound DNA in 0.5M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, which can either be used as a probe for OFF1, or which encodes functionally equivalent gene product; and

(vii) a DNA sequence that hybridizes to the sequences of (i) to (iv) under moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C yet which still encodes a functionally equivalent gene product.

30. (NEW) An expression vector comprising the DNA sequence of Claim 29.

31. (NEW) An expression vector according to Claim 30, being a plasmid.

32. (NEW) A genetically engineered host cell containing the DNA sequence of Claim 29, operatively associated with a regulatory element heterologous to the DNA sequence which directs the expression of the DNA sequence by the host cell.

33. (NEW) An amino acid sequence coded by the nucleic acid sequence of Claim 29.

34. (NEW) A DNA sequence which is complementary to at least a portion of any one of the sequences of Claim 29, capable of being transcribed to mRNA which is an anti-sense to at least a portion of the mRNA transcribed by any one of the sequences of Claim 29, said portion being sufficient to inhibit translation of the mRNA to protein.

35. (NEW) An anti-sense mRNA sequence transcribed from the DNA of Claim 34.

36. (NEW) A pharmaceutical composition comprising the expression vector of Claim 31.

37. (NEW) A pharmaceutical composition comprising the amino acid sequence of Claim 33.

38. (NEW) A pharmaceutical composition according to Claim 36, for immunization against cancer.

39. (NEW) A pharmaceutical composition according to Claim 37, for immunization against cancer.

40. (NEW) A pharmaceutical composition according to Claim 38, for immunization against breast cancer.

41. (NEW) A pharmaceutical composition according to Claim 39, for immunization against breast cancer.

42. (NEW) A pharmaceutical composition according to Claim 36, for the treatment of transplant rejections, autoimmune

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diseases, pathological pregnancies and for enhancing fertilization rates during IVF treatment.

43. (NEW) A pharmaceutical composition according to Claim 37, for the treatment of transplant rejections, autoimmune diseases, pathological pregnancies and for enhancing fertilization rates during IVF treatment.

44. (NEW) A pharmaceutical composition according to Claim 36, for use as a growth factor of bone-marrow progenitor cells.

45. (NEW) A pharmaceutical composition according to Claim 37, for use as a growth factor of bone-marrow progenitor cells.

46. (NEW) A pharmaceutical composition according to Claim 44, wherein the cells are granulocyte monocytes.

47. (NEW) A pharmaceutical composition according to Claim 45, wherein the cells are granulocyte monocytes.

48. (NEW) A growth factor for bone marrow progenitor cells comprising as an active ingredient the amino acid sequence of Claim 33.

49. (NEW) An expression vector comprising the DNA of Claim 34.

50. (NEW) A pharmaceutical composition comprising the expression vector of Claim 49.

51. (NEW) A pharmaceutical composition comprising the anti-sense mRNA sequence of Claim 34.

52. (NEW) A pharmaceutical composition according to Claim 50, for the treatment of cancer.

53. (NEW) A pharmaceutical composition according to Claim 51, for the treatment of cancer.

54. (NEW) A pharmaceutical composition according to Claim 52 for the treatment of breast cancer.

55. (NEW) A pharmaceutical composition according to Claim 53 for the treatment of breast cancer.

56. (NEW) A pharmaceutical composition according to Claim 50, for the induction of abortion.

57. (NEW) A pharmaceutical composition according to Claim 51, for the induction of abortion.

58. (NEW) A method for the diagnosis of cancer comprising: detecting elevated to levels of mRNA transcribed from DNA sequences depicted in Fig. 1 or Fig. 4.

59. (NEW) A method according to Claim 58, wherein the cancer is selected from the group consisting of: breast cancer, hepatoblastoma, leukemia, Hodgkin's and non-Hodgkin's lymphomas and embryonal tumors.

60. (NEW) A method for the detection of Downs' Syndrome, comprising detecting elevated levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.

61. (NEW) A method for the detection of pathological

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pregnancies comprising detecting decreased levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.

62. (NEW) A method according to Claim 61, wherein the pathological pregnancy is selected from the group consisting of: spontaneous abortion and miscarriage, premature contractions, toxemia, premature delivery.

63. (NEW) A method according to Claim 58, wherein the level of the DNA expression is detected using AT-PCR.

64. (NEW) A method for isolating the DNA sequence of Fig. 1 or 4, substantially as hereinbefore described.

65. (NEW) A method for the treatment of an individual in need of such treatment comprising: administering to a subject in need of such treatment a therapeutically effective amount of the expression vector of Claim 31.

66. (NEW) A method for the treatment of an individual in need of such treatment comprising: administering to a subject in need of such treatment a therapeutically effective amount of the amino acid sequence of Claim 33.

REMARKS

Claims 29-66 presently appear in this case. The above amendments to the claims are being made in order to place the claims into better condition for examination.

In re appln. of C.MOROZ (MOROZ3)

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: 

Roger L. Browdy
Registration No. 25,618

RLB:edg

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Facsimile No.: (202) 737-3528

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#4/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
MOROZ, C.)	
)	
IA No.: PCT/IL99/00485)	
)	Washington, D.C.
IA Filed: 8 September 1999)	
)	
U.S. App. No.:)	
(Not Yet Assigned))	
)	March 12, 2001
National Filing Date:)	
(Not Yet Received))	
)	
For: DNA SEQUENCE ENCODING...)	Docket No.: MOROZ3

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and
prior to calculation of the filing fee, kindly amend as
follows:

IN THE SPECIFICATION

After the title please insert the following
paragraph:

REFERENCE TO RELATED APPLICATIONS

The present application is the national stage under 35
U.S.C. 371 of international application PCT/IL99/00485, filed
September 8, 1999, which designated the United States, and
which international application was published under PCT Article
21(2) in the English language.

IN THE CLAIMS

Please cancel original claims 10, 12, 13, 19, 21 and 27 without prejudice.

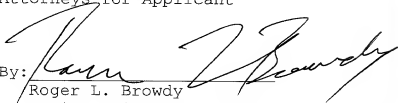
REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to reduce the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By:


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Registration No. 25,618

RLB:edg

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Facsimile No.: (202) 737-3528

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09736637-082104

* DNA SEQUENCE ENCODING ONCOFETAL FERRITIN PROTEIN

FIELD OF THE INVENTION

This invention relates to novel DNA sequences, amino acid sequences coded by them, detection method using said DNA sequences and pharmaceutical composition.

5 BACKGROUND OF THE INVENTION

Iron is known to be an essential element of the makeup of every living organism, but may also become toxic at physiological pH values by virtue of its tending to oxidize, hydrolyze and precipitate as insoluble ferric oxide polymers. The protein ferritin, found in all living cells, is the body's means
10 for ensuring that iron toxicity does not occur. Ferritin functions by storing iron in the cells in a soluble and readily available form. The iron stored in cells may then be mobilized whenever needed by the body, for example for erythropoiesis.

The name "*ferritin*" actually encompasses a number of individual
15 isomeric forms which are characteristic of different tissue types. Each isoform has 24 subunits of two distinct types, being light subunits (L) and heavy subunits (H). These subunits differ in molecular weight, the light subunit being about 18 kDa, and the heavy subunit about 19-21 kDa. The isoforms extracted from different tissues or organs typically exhibit

different isoelectric points, with the isoelectric focusing pattern of human tissues forming a continuous spectrum: those tissues associated with high iron storage have ferritins at the basic end of the spectrum (e.g. spleen and liver), while iron poor tissues, (e.g. heart and placenta) and malignant cells have acidic ferritins. (Drysdale, *Ciba Found. Symp.*, 51:41, 1977). The difference in isoelectric point appears to be related to the different distribution of light and heavy subunits in each type. Specifically, heavy subunit-rich ferritins are relatively acidic, and light chain rich ferritins are relatively basic (Cosell, *et al.*, in *Ferritins and Isoferritins as Biochemical Markers*, p. 49-65, 1984, Elsevier). Current studies indicate that the H and L subunits are encoded by a complex group of genes.

A specific type of acidic isoferritin has been shown to be characteristic of neoplastic cells and placental cells (Drysdale and Singer, *Cancer Res.*, 44:3352, 1974). This protein is also known as oncofetal ferritin or placental isoferritin (PLF). Human placental ferritin has been shown to be composed predominantly of a single subunit type comigrating with a liver ferritin standard on SDS-PAGE (Brown *et al.*, *Biochem. J.*, 182:763, 1979). However, an immunoradiometric assay performed with anti-human spleen ferritin has shown tissue specific antigenicity for PLF (Brown *et al.*, *supra*). A three subunit structure has been revealed for PLF (Moroz *et al.*, *G.I. Pat. Clin.*, 1:17-23, 1986). In addition to the L and H subunits characteristic of all ferritins, there is also a high molecular weight (43 kDa) subunit which appears to be unique for human placenta, and thus provides a potential site for identification of the placental isoferritin molecule as distinguished from any other type of ferritin.

Various ferritin isoforms have been isolated from normal and malignant tissues, the most acidic ones predominating in tumor and fetal tissues (Drysdale, 1976. *Ciba Found. Symp.* 51:41; Arosio *et al.*, *J. Biol. Chem.*, 253:4451, 1978). It has been suggested that the assay of acidic

isoferritin in the serum may be of value in the diagnosis of malignancy (Hazard *et al.*, *Nature*, **265**:755, 1977). Elevated concentrations of serum ferritin were found in patients suffering from a variety of malignant diseases, including acute lymphocytic leukemia (ALL) (Matzner *et al.*, *Am. J. Hematol.*, **2**:13, 1980), hepatoma (Giannoulis, *Digestion*, **30**:236, 1976) and recently Hodgkin's disease (Bezwoza *et al.*, *Scand. J. Haematol.*, **35**:505, 1985). In assays based on antibodies against HeLa cell ferritin, Hazard and Drysdale found higher concentrations of ferritin in sera from patients with various tumors than in the same sera assayed by antibodies directed against normal liver ferritin (Hazard, *et al.*, *supra.*). Others have failed to demonstrate a consistent pattern of isoferritins in tumor tissues (Cragg *et al.*, *Br. J. Cancer*, **35**:635, 1977; Halliday, *et al.*, *Cancer Res.*, **36**:4486, 1976) or in sera obtained from patients with tumors (Jones, *et al.*, *Clin. Chim. Acta.*, **85**:81, 1978; Jones, *et al.*, *Clin. Chim. Acta.*, **106**:203, 1980).

Although publications concerning the existence of oncofetal ferritin or placental isoferritins have been evident at least since the year 1976, up until today the sequence of this protein and the gene encoding therefor were not known. This is probably due to the fact that the protein itself is hydrophobic and almost devoid of iron and as a consequence extremely sticky and not capable of sedimentation even by high speed centrifugation thus hindering its isolation and purification. In addition, the sequence of the gene coding for oncofetal ferritin could not be found in regular cDNA libraries, probably due to the fact that its expression in these libraries is extremely low. The protein is secreted only by the placenta during pregnancy or by cancer cells in malignant diseases such as lymphoproliferative disorders, breast cancer and in HIV infection.

Breast cancer is a malignant disease effecting different populations at a rate of one to every 9-13 of women. Early diagnosis of breast cancer is known to considerably improve the prognosis of the patient. Diagnosis of breast

cancer is based today mainly on imaging techniques, such as mammographs verified at times by biopsies. Blood-based assays of breast cancer have been reported in the literature, for example, biomarkers such as CA 15.3. (Daly, L. *et al.*, *Comparison of a novel assay for breast cancer mucinto and CA 54 15.3* 5 *carcinoembryonic antigen*, *J. Clin. Oncol.*, **10**:1057-65); the CA 549(2) marker (Dermers, I.M., *et al.*, CA 549: a new tumor marker for patients with advanced breast cancer *J. Clin. Lab. Anal.*, **2**: 168-73, 1988); and the marker CA M29 CEA (Duistrian, A.M., *et al.*, *Evaluation of CA M26, CA M29, CA 15.3 and CEA as circulating tumor markers in breast cancer patients*. *Tumor* 10 *Biol.*, **12**:82-90, 1991). However these assays, reported in the scientific community have not gained, to date, clinical significance (Werner, M., *et al.*, *Clinical utility and validation of emerging biochemical markers for mammary adenocarcinoma*. *Clin. Chem.*, **39**/11(B):2386-96, 1993).

U.S. 4,882,270 discloses an assay for the detection of breast cancer 15 based on determination of oncofetal ferritin. The assay is based on binding of the oncofetal ferritin to specific monoclonal antibodies.

Pathological pregnancy is a term commonly used to describe a multitude of symptoms which create difficulties in carrying a child to term and include spontaneous abortion and miscarriage, premature contractions, 20 toxemia, premature delivery. U.S. 4,954,434 discloses the fact that low levels or absence of PLF in pregnant women can serve as a marker for potentially high risk pregnancy. Detection of this state is again achieved by monoclonal antibodies which has PLF specificity. This patent also concerns treatment and prevention of actual and potentially pathological pregnancy by the 25 administration of this protein. However, since the sequence of the protein was not known at the date of the patents, the treatment suggested involved administration of partially purified protein and not of recombinant pure proteins.

U.S. Patents 5,571,678, 5,120,640 and 5,283,177 are all directed to methods for assaying the presence and evaluating the prognosis of acquired immunodeficiency associated with HIV induction, by determining levels of placental isoform of ferritin by monoclonal antibodies.

5 All the above detection methods concern antibody-based assays. While
* such assays are known to be useful in conditions where the level of the
 protein to be detected is quite high, they are notorious for eliciting a
 false-negative answer where the protein level is low. Against this, assays
 based on amplification of mRNA (RT-PCR) are much more sensitive and can
10 detect even minute expression of mRNA. Thus there is need today for a
 RT-PCR method for detection of oncofetal ferritin for detection of breast
 cancer and for diagnosis of high risk pregnancies at its early stage.

 Furthermore, it would have been desirable to provide pure oncofetal
 ferritin protein prepared by recombinant processes for therapeutic and
15 vaccination purposes.

SUMMARY OF THE INVENTION

 The present invention is based on the finding of the sequence of the
 oncofetal ferritin gene and of the oncofetal ferritin protein. The inventor was
 the first to discover the full sequence of the gene that codes for the protein
20 termed hereinafter as "*oncofetal ferritin I (OFF1) protein*".

 Thus, the present invention concerns a DNA sequence coding for the
 subunit of the oncofetal ferritin protein termed "*oncofetal ferritin I*" (OFF1)
 protein selected from the group consisting of:

- (i) a DNA sequence as depicted in Fig. 1;
- 25 (ii) a DNA sequence as depicted in Fig. 4;
- (iii) a DNA sequence which codes for the same amino acid
 sequences encoded by the sequence of (i) or (ii);

- (iv) fragments of any of the sequences of (i) to (iii) that code for a functionally equivalent gene product;
- (v) a DNA sequence that has at least-80% homology, as determined by hybridization under stringent conditions, to any one of the sequences of (i) to (iv) and code for a physiologically active protein.
- (vi) a DNA sequence that hybridizes to the sequences of (i) to (iv) under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, (Ausubel F.M. *et al.* eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Assoc., Inc. and John Wiley & Sons., Inc. New York at p. 2.10.3), which can either be used as a probe for OFF1, or which encodes functionally equivalent gene product; and
- (vii) a DNA sequence that hybridizes to the sequences of (i) to (iv) under moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, supra), yet which still encodes a functionally equivalent gene product.

The sequence of Fig. 1 was isolated from a cDNA library obtained from breast cancer patients while the DNA sequence of Fig. 4 was obtained when using PCR amplification where the sequence of Fig. 1 was used as a template. The two sequences differ only in the 5' non-coding region which include 2 single nucleotide substitutions as well as a single base insertion and one deletion.

The DNA sequences of the invention also include DNA sequences which code for the same amino acid sequences as those of Figs. 1 or 4. It is known that due to the degenerative nature of the genetic code, a large number of alternative DNA sequences, may code for the same proteins. Thus all

sequences which code for the same amino acid sequences are encompassed by the scope of the invention.

The present invention further concerns DNA sequences having at least 80% homology either to the DNA sequence of Fig. 1 or 4, or to the DNA sequences coding the same types of amino acid sequences, the homology determined by hybridization under stringent conditions. Examples of highly stringent conditions are given in (vi) above and of moderately stringent conditions are given in (vii) above. The artisan will appreciate the fact that there exists a large number of sequences capable of hybridization under such conditions, some of which code for more physiologically active OFF1 proteins than others. Those sequences which fall under the scope of the invention, are those which code for a functionally equivalent gene product, as will be explained hereinbelow.

The present invention also encompasses DNA sequences that hybridize to the sequences of Figs. 1 or 4, or to the cDNA inserts contained in the deposited cells, under highly stringent conditions, as specified above. Such DNAs can be used as probes to detect the OFF-1 gene or mRNAs (e.g. by hybridization or PCR amplification assays). Alternatively DNAs that hybridize under highly stringent or less stringent conditions (specified above), yet which encode a functionally equivalent gene product are also encompassed by the invention.

The present invention further concerns fragments of all the above sequences, which code for an OFF1 protein having functionally equivalent gene product will be explained hereinbelow.

The term "*functionally equivalent gene product*", refers to an amino acid sequence, which is physiologically active in a manner similar to that of the native OFF1 protein. Such an activity can be tested, for example, by determining the immunosuppressive activity in cell mediated immunity, as is well known in the art.

The present invention further concerns expression vectors comprising said DNA sequences, as well as a host cells transfected with such expression vectors. Expression may be obtained in any suitable pro-or eucaryotic expression systems using known methods e.g. as described in Genentech EP 200341.

Suitable expression vectors are DNA sequences encoding OFF1 and operably linked to suitable control sequences capable of effecting the expression of OFF1 in the host. Such control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control termination of transcription and translation. For expression of OFF1 in eukaryotic cells, the vector also should include DNA encoding a selection gene.

Vectors include plasmids, viruses (including phages) and integratable DNA fragments, i.e. fragments that are integratable into the host genom by recombination.

Preferred host cells are cells derived from multicellular organisms. In principle, any higher eucaryotic cell culture is workable whether from vertebrate or invertebrate culture. Examples useful in host cell lines are Chinese Hamster Ovary (CHO) cell lines and COS 7 cell lines.

In another embodiment of the present invention, the cells and tissues may be engineered to express an endogenous gene under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional

initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals, mRNA stability
5 elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence,
10 e.g., inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has
15 broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous
20 DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a
25 correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes simplex virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin *et al.*; International Application No. PCT/US92/09627 (WO93/09222) by Selden *et al.*; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultschi *et al.*, each of which is incorporated by reference herein in its entirety.

The present invention of course concerns also a recombinant protein coded by the above DNA sequence. Said recombinant protein is in fact the first recombinant OFF-1 protein produced, and enables production of large amounts of such protein in a pure form for therapeutic and detection purposes as will be explained hereinbelow.

The present invention further concerns anti-sense RNA sequences, which are complementary to the mRNA sequences transcribed from the above DNA sequences, and thus are capable of neutralizing the expression of native OFF-1 gene in cells. The present invention further concerns DNA sequences coding for said anti-sense mRNA, as well as expression vectors comprising said DNA sequences.

The present invention also concerns pharmaceutical compositions of two types.

According to the first aspect of the invention, termed "*the OFF1 activating aspect*" the pharmaceutical compositions of the invention comprise DNA sequences coding for the OFF1 protein, expression vectors comprising said DNA sequences, for expression in specific target cells, or the recombinant OFF1 protein itself. The above agents may be used in the immunization of a subject against diseases which are manifested by abnormally high expression of the OFF1. Examples of such diseases are: cancer, in general, and breast cancer and lymphomas in particular as well as HIV infections.

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The pharmaceutical composition according to the activating aspect of the invention may be used, as such, for increasing the level of the OFF1 protein for the treatment of conditions manifested by lower than normal levels of the OFF1 protein. If the level of the OFF1 protein can be raised again to
5 normal level, either by administering to the subject recombinant OFF1 protein, or by transfecting the subject's target cells with an expression vector comprising the DNA coding for the OFF1 protein, then the pathological conditions may be alleviated. Pathological conditions treated by these pharmaceutical compositions are pathological pregnancies manifested by
10 spontaneous abortion and miscarriage, premature contractions, toxemia and premature delivery. In addition, the pharmaceutical compositions may be used to inhibit transplant rejection, for example, specific T-cell mediated immunity like that of a mother against her embryo.

Using the same principal, the pharmaceutical compositions of the
15 invention may also be used for the treatment, alleviation, or prevention of autoimmune diseases, such as: Coeliac disease, Rheumatoid arthritis, and Multiple Sclerosis which are T-cell mediated autoimmune diseases.

By another option, the pharmaceutical composition of the activation aspect of the invention may be used to support normal pregnancies, for
20 example, for increasing the chances of success of *in vitro* fertilization (IVF), in both human and non human subjects.

According to another surprising finding it was found that OFF1 can serve as a growth factor for bone marrow progenitor cells. Thus the pharmaceutical composition of the invention may be used to enhance growth
25 of bone marrow progenitor cells, for example, where due to some pathological condition their number has decreased. Such a condition occurs, for example, in patients treated by mega doses of cytotoxic drugs which kill bone marrow cells such as cancer patients, HIV infected patients and the like. In addition the pharmaceutical compositions of the inventions could be used

in patients who need bone marrow replacements such as those with genetic metabolic defects or autoimmune diseases.

By another alternative the DNA coding for OFF1 can be used in the development of a chimera which will enable further grafting of organ allografts or xenografts identical with the bone marrow donor.

In addition, a preparation comprising OFF1 can be used *ex vivo* as a growth factor for bone marrow progenitor cells, for example, from bone marrow obtained from a donor prior to implantation.

An example of bone marrow progenitor cells are granulocyte monocyte progenitor cells.

By another aspect of the invention, termed "*the OFF-1 neutralizing aspect*" the pharmaceutical compositions of the invention comprise anti-sense mRNA to the OFF-1, expression vectors comprising DNA sequences coding for said anti-sense mRNA, or antibodies against the OFF-1 protein. The neutralizing aspect of the invention, is intended to lower the levels of OFF-1, where it is abnormally high as compared to normal tissue, notably for the treatment of cancer, especially breast cancer.

By another option the neutralizing aspect of the invention may be used in order to reduce the normal level of OFF1 which is required to maintain a pregnancy to term and thus cause abortion (Moroz, C., 9th International Congress of Immunology, San Francisco, 1995).

The present invention further concerns a method for the detection of cancer, or for the evaluation of the prognosis of a cancer patient, by determining the level of the OFF1 gene expression in said patients.

U.S. Patent 4,882,270 discloses a method for detecting breast cancer, by using antibodies against isoferritin placental protein.

This method detects said protein in early stages of the cancer only on lymphocytes not in the serum. The protein can be detected in the serum only at a very late stage of the disease when the tumor has already metastasized.

The problem with lymphocyte-based detection, is double fold: first, technical issues concerning detection are quite severe, since there is a requirement to isolate fresh lymphocytes from the blood and assay within hours without an opportunity to retest at a later date. This severe technical problem prohibits the use of this method of detection in widely used screening assays. Second, during progressive stages of cancer, the number of positive lymphocytes decreases dramatically, and thus it is not possible to detect the cancer, using lymphocytes directed anti-ferritin antibodies. Thus, according to said U.S. patent, it is possible to detect cancer at its early stage, where the number of lymphocytes is high, as well as at the very last stages, where the level of the isoferrin placental protein is so high that it is shed to the serum, but in most stages of the cancer, the low number of positive lymphocytes avoids detection of cancer.

Against this, the detection methods of the present invention, are based on RNA amplification, and are sensitive enough to detect even slightly elevated levels of the OFF1 mRNA present in small amounts of the patient's blood in virtually all stages of cancer. The method of the invention detects the mRNA in circulating cancer cells, whereas the protein is shed from tumors and binds to lymphocytes.

In addition to detection of the presence of cancer, the level of OFF1 protein is also a good indicator of the prognosis of cancer, for example the change in mRNA level after removal of the tumor by surgery or chemotherapy may indicate disease prognosis.

Examples of cancers which can be detected by the methods of the invention are breast cancer, hepatomas, leukemias, lymphomas and embryonal tumors such as neuroblastoma and hepatoblastoma.

In addition, elevated levels of OFF1 expression, are typical of Down Syndrome. In Down Syndrome there are elevations of embryonal proteins like α -fetoprotein (AFP). Also the Syndrome is associated with decreased

immunoreactivity and high incidence of cancer. Thus by detecting high levels of this protein it is possible to determine also Down's disease.

By another embodiment, the present invention concerns a method for the detection of diseases connected with pathological pregnancies, comprising
5 detecting a lower level than normal of the OFF-1 expression. The term
"pathological pregnancies" groups together a large number of disorders including spontaneous abortion and miscarriage, premature contractions, toxemia, premature delivery.

The detection of the level of the OFF-1 expression, both for
10 determining higher than normal levels (various types of cancer) or lower than normal levels (various types of pathological pregnancies) can be carried out by utilizing reverse transcriptase polymerized chain reaction (RT-PCR). This method, considerably amplifies the OFF-1 mRNA present in the blood enabling its detection, even in minute levels.

15 The present invention further concerns a method for the isolation of the cDNA of the invention as specified in Fig. 1 or 4 as will be appreciated in the "Detailed Description" section of the specification.

The present invention further concerns primers for use in the above isolation method. These primers may also be used in RT-PCR, for the
20 detection purposes of the invention. The primers are selected from the group consisting of:

5' GGT GGC GAC GAC TCC TGG AGC CCG 3'
5' TTG ACA CCA GAC CAA CTG GTA ATG 3'
5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'
5' GAT AGG ATC TTT AGC GAC AGC CGA 3'
5' ATG GCG GCC TCT GAG TCC TGG TGG 3'
5' CGG GCT GAA TGC AAT GGA GTG TGC 3'
5' GAC CCC CAT TTG TGT GAC 3'
5' CGA CGA CTC CTG GAG CCC G 3'
5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'
5' AGC CGA CAG CGA TTT CTA GGA TAG 3'
5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'
5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'
5' CAG ACG TTC TTC GCC GAG AGT CGT 3'
5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'
5' CAT TTC GGG GAT TCG GGG GA 3'
5' GGG GGA CGG AAC CCG GCG CT 3'
5' CCC TCT ACA CTT ATC ATC TTC 3'
5' CTA TCC TAG AAA TCG CTG TCG GCT 3'
5' GTC ACT ACT GGA ATT CCC TTC TCC 3'
5' GGA GAA GGG AAT TCC AGT AGT GAC 3'
5' GGA AAT CGC TGT CGC CTA ACC 3'
5' GGT TAG GCG ACA GCG ATT TCC 3'
5' GGC CAC GCG TCG ACT AGT AC 3'
5' GTA ATG CAC ACTCCA TTG GC 3'
5' GTA ATG CAC ACT CCA TTG 3'
5' GCG CTC AGC TGG AAT TCC 3'
5' GGA ATT CCA GCT GAG CGC 3'
5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3'
5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'
5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'
5' CCC GCT CGA GTC AGG GTG ACC GAA AAA TCA G 3'

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BRIEF DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows the nucleic acid sequence of clone T16 isolated from T47D breast cancer cDNA library. Initiation and termination codons of the open reading frame are indicated by dark bars;

Fig. 2A shows a comparison of the nucleic acid sequences (upper sequence) of clone 4.7 isolated from a placenta cDNA library exhibiting normal human FTH, and the sequences (lower sequence) of clone T16 isolated from human breast cancer T47D cDNA library. Initiation and termination codons of the open reading frame are marked by dark boxes;

Fig. 2B shows in a schematic representation the comparison of the two sequences shown in Fig. 2A. Differences in nucleic acid sequences are represented by the shaded areas;

Fig. 3 shows a comparison of sequence homology between cDNA clone T16 and human mitochondrial cytochrome oxidase I DNA;

Fig. 4 shows a comparison of nucleic acid sequences between placental cDNA obtained by PCR amplification using T16 specific primers (upper sequence) and T16 cDNA sequence obtained from the T16 cDNA clone (lower sequence). Identical nucleic acid sequences are indicated by a dotted line. Initiation and termination codons are indicated by a dark bar;

Fig. 5 shows the nucleic acid sequence and deduced amino acid sequence of the cDNA of OFF1;

Fig. 6A shows the relative expression of FTH in mRNA and OFF1 RNA among total mRNA isolated from different tissues and optimized with β -actin expression:

Fig. 6B shows the relative expression of FTH mRNA hybridized with 32^PPstI 3' fragment of liver FTH cDNA (530 bp), from either normal HBL human lactating breast or from cancer cell lines (MCF-7 and T47D);

Fig. 7 shows the sequence of clone T16. primers used for PCR are indicated in the above sequence;

Fig. 8 shows the restriction enzyme map sequence of clone T16;

Fig. 9A shows SDS-PAGE (12%) of total cell lysates (lane 2) 10 μ l from *E.coli* containing the vector pGEX alone or the pGEX constructed to contain C48 fragment (lane 4) or total constructs containing full length OFF1 (lane 6). The recombinant proteins are marked by arrows;

Fig. 9B shows the same as Fig. 9A but reactive with CMH-9 in antibodies (lanes 4 and 6 indicating presence of protein);

Fig. 10 shows the effect of the protein of the invention on CFU-GM colony formation obtained from three healthy donors;

Fig. 11 shows the effect of the protein of the invention and its combination with GM-CSF or CMH9 Moab on CFU-GM colony formation; and

Fig. 12 shows a scheme summarizing the effect of OFF1 on immunoregulation.

DETAILED DESCRIPTION OF THE INVENTION

EXPERIMENTAL PROCEDURES

Example 1 Cloning of OFF1 cDNA

λ gt11 cDNA libraries were prepared using total poly A⁺RNA from human breast cancer cell line T47D or from human placenta. Both libraries were randomly primed from total poly (A)⁺RNA. EcoRI linkers were attached to the cDNAs which were inserted into the EcoRI site of the bacteriophage λ gt11.

About 10^6 plaque forming units were screened by using human liver ferritin (FTH) cDNA provided by R. Cortese (Constanzo *et al.* *EMBO*, 3:23-27, 1984). Plaque hybridization was carried out according to Berton, W.D. and Davis R.W., (*Screening λ gt recombinant clones by hybridization to single plaques in-situ*, *Science*, 196:180-182 (1997)). In brief, plaque hybridization was carried out at 42°C, 5xSSC and subsequent washing 3 times with 2xSSC 0.1% SDS at room temperature and twice with 1xSSC, 0.1% SDS at 68°C.

Clones were isolated that gave hybridization signals after two rounds of screening. PCR amplification was performed on clones using one primer from the λ gt11 vector (λ gt11 F-1060 or λ gt11 R-1061, Table 1) and one FTH gene-specific primer (either 17R to amplify toward the 5' end of 17F to amplify toward the 3' end: as indicated in Table 1) as described above, PCR amplification was performed for 30 cycles under standard conditions. PCR products from the clones derived from both the 5' and the 3' end of the cDNA clone were selected according to their size, so that their sequence would produce a contig of maximum length. PCR products were purified by Qiagen PCR purification columns according to the manufacturer's instructions and were sequenced using standard protocols for the ABI373 or 377 primer mated sequencer with the 1060 and 1061 primers (Table 1) and specific primers until the full sequence was determined.

Table 1
List of Primers

Name	#MR	Sequence		
1060F	24	5' GGT GGC GAC GAC TCC TGG AGC CCG 3'	75%	
1061R*	24	5' TTG ACA CCA GAC CAA CTG GTA ATG 3'	45.80%	
17F	27	5' GAC CGC GAT GAT GTG GCT TTG AAG AAC 3'	52%	27618
X1.1F	24	5' GAT AGG ATC TTT AGC GAC AGC CGA 3'	50%	24880
X.1.1R	24	5' ATG GCG GCC TCT GAG TCC TGG TGG 3'	67%	
2.1F	24	5' CGG GCT GAA TGC AAT GGA GTG TGC 3'	58%	
3.4F	18	5' GAC CCC CAT TTG TGT GAC 3'	55.50%	
1060F/S	19	5' CGA CGA CTC CTG GAG CCC G 3'	73.70%	
1061r/Bio	24	5' Biotin-TTG ACA CCA GAC CAA CTC GTA ATG 3'	45.80%	
16X.1R	24	5' AGC CGA CAG CGA TTT CTA GGA TAG 3'	50%	24879
17R	27	5' GTT CTT CAA AGC CAC ATC ATC GCG GTC 3'	52%	27385
3'COD R	28	5' GCT TTC ATT ATC ACT GTC TCC CAG GGT G 3'	50%	28313
5' NCF	24	5' CAG ACG TTC TTC GCC GAG AGT CGT 3'	58%	24870
4869	27	5' CAG ACG TTC TTC GCC GAG AGT CGT CGG 3'	63%	
NFG	20	5' CAT TTC GGG GAT TCG GGG GA 3'	60%	
NFGP-2	20	5' GGG GGA CGG AAC CCG GCG CT 3'	80%	201880
767-F	21	5' CCC TCT ACA CTT ATC ATC TTC 3'	43%	211616
16-F	24	5' CTA TCC TAG AAA TCG CTG TCG GCT 3'	50%	241173
ECO-F	24	5' GTC ACT ACT GGA ATT CCC TTC TCC 3'	50%	24960
ECO-R	24	5' GGA GAA GGG AAT TCC AGT AGT GAC 3'	50%	24961
SPF	21	5' GGA AAT CGC TGT CGC CTA ACC 3'	57%	211667
SPR	21	5' GGT TAG GCG ACA GCG ATT TCC 3'	57%	211668
AUAP	20	5' GGC CAC GCG TCG ACT AGT AC 3'	65%	202738
NC-F	20	5' GTA ATG CAC ACTCCA TTG GC 3'	50%	203814
SNC-F	18	5' GTA ATG CAC ACT CCA TTG 3'	44%	181897
BNC-F	18	5' GCG CTC AGC TGG AAT TCC 3'	55.50%	181898
BNC-R	18	5' GGA ATT CCA GCT GAG CGC 3'	61.10%	181905
pGEX-F	29	5' GTG GGA TCC CCA TGA CGA CCG CGT CCA CC 3'	67%	29391
pGEX-R1	27	5' GAC TCG AGT TAA GCC GAC AGC GAT TTC 3'	51.85%	27578
pGEX-R2	29	5' GAC TCG AGT CAG GGT GAC CGA AAA ATC AG 3'	51.70%	29396
pGEX-R3	31	5' CCCGCTCGAGTCAGGGTGACCGAAAAATCAG 3'	58%	31277

09785857-082101

Example 2 Sequence analysis

The cDNA sequences and its deduced protein sequence were used to search the complete combined Gene Bank/EMBL database and the complete Swiss Prot database with BLAST and FASTA programs, respectively.

Example 3 RNA preparation and northern blot analysis

Total RNA was extracted from cells by guanidinium isothiocyanate solubilization and prepared by phenol-chloroform extraction. PolyA⁺ RNA was purified using 2 rounds of oligo(dT) chromatography. Total RNA (25 µg) was separated by electrophoresis through 1% agarose. 2.2 M formaldehyde gels, transferred to nylon membranes (Hybond-N, Amersham, Arlington Heights, IL) and fixed by baking at 80°C for 2 hrs. The probes used for hybridization were:

- (1) PstI 3' fragment of FTH cDNA (530 bp);
- (2) T16 specific SPF-16R PCR cDNA product.

After hybridizations with ³²P-labeled probes (10⁶ cpm ml) 42°C, 50% formamide 5xSSC, blots were washed in 2xSSC, 0.1% SDS at 25°C, followed by 0.1xSSC, 0.1% SDS at 55°C.

- (20) Human tissues mRNA was purchased from Clontech (Cat. # 7760-1) and hybridized according to the manufacturer's instructions.

Example 4 Detection of OFF1 transcripts by RT-PCR in peripheral blood

25

1. Preparation of blood derivatives

- Tube A. To whole blood (0.5 ml) 1 ml red blood cell lysis buffer (Boehringer) was added at room temperature and mixed by inversion (without vortex). The preparation was then stored for 10 mins. at room temperature with agitation, and

30

centrifuged at 2,500 rpm for 5 mins. at room temperature. The pellet was then washed with PBS.

Tube B 4.5 ml whole blood were centrifuged for 5 mins. at room temperature. at 1,030 g. In order to buffy coat, about 0.5 ml
5 „ lysis buffer 1 ml was added for 10 mins. at room temperature. The mixture was placed on a shaker, and then centrifuged at 2,500 rpm for 5 mins. at room temperature. The pellet was finally washed with PBS.

Tube C To 5 ml of whole blood 10 ml of PBS were added.
10 Mononuclear cells were isolated by density centrifugation on Ficoll hypaque (5 ml), and spun at 450 g for 25 mins. at room temperature. The pellet was washed with PBS (resulting in about 5×10^6 lymphocytes).

15 II. Isolation of RNA (according to Tri-reagent protocols supplied by the manufacturer)

1. Cells were lysed with Tri Reagent; To Tube A - 0.5 ml were added, to Tubes B and C 1 ml was added by repetitive
20 pipetting.
2. The preparation was stored for 5 mins. at room temperature, and then 0.2 ml chloroform per 1 ml of Tri Reagent were added and vortexed for 5 secs. The resulting preparation was stored for 2-15 mins. at room temperature and centrifuged
25 12,000 g 15 mins. at 4°C.
3. The aqueous phase was transferred to a fresh tube, and precipitated by 0.5 ml isopropanol per 1 ml TRI Reagent on ice for 5-10 mins. (or at room temperature) and centrifuged 12,000 g at 4°C.

4. The supernatant was removed and the RNA pellet was washed with 75% ethanol by vortexing and centrifugation.

The RNA pellet was air dried and then dissolved in 20 μ l DEPC- water. RNA has O.D. at 260/280 = 1.6-1.9 yield = 2.5 μ g (for Tubes B and C).

III. RT-PCR

1 μ g RNA (or 14 μ l whole blood RNA of Tube A) was placed in total volume of up to 15 μ l in DEPC water, heated at 70°C for 10 mins. and cooled immediately on ice. Then it was spun briefly and 10 μ l of mix was added as follows: 5 μ l M-MLV RT 5xReaction buffer; 1 μ l dNTPs (12.5 μ l); 1 μ l Recombinant Rnasin Ribonuclease (20 u); 2 μ l DEPC water; 1 μ l M-MLV Reverse transcriptase (RT) (200 units).

The mixture was incubated for 60 mins. at 37°C followed by 10 mins. at 70°C to stop the reaction.

PCR

For PCR the following reagents were used: 1 μ l cDNA (or 0.5 μ l cDNA for PCR of normal ferritin (FTH) H (Chain); 5 μ l 10x reaction buffer for DNA polymerase; 1 μ l forward primer (~ 10 pmole); 1 μ l reverse primer (~ 10 pmole); 1 μ l dNTPs (12.5 mM), 0.5 u Taq polymerase from Appligen (0.1 μ l); Takara 0.2 gel DDW water up to 50 μ l.

The PCR program (cycles) was as follows:

- | | | |
|------------------|-------------|-------------|
| 1) 94° - 2' | 2) 50° - 2' | 3) 72° - 1' |
| 4) 94° - 1' | 5) 50° - 2' | 6) 72° - 3' |
| 7) 29 times to 4 | 8) 94° - 1' | 9) 50° - 2' |
| 10) 72° - 10' | 11) 4° | |

PCR products were identified by electrophoresis on 1% agarose gel and 0.10 µg ethidiumbromide.

Since T16 transcripts do not yield bands after the first PCR, in order to amplify the results the initial PCR is followed by nesting PCR, using 1 µl of PCR 1 diluted 1:100 in the above PCR program.

As an example, the following primers were used for nesting PCR

PCR1: XIF → 16R

nesting PCR 2: 17F → SPR

For normal ferritin: 17F → 3'R yields a visible product.

No nesting is required.

RESULTS

Example 5 Isolation of cDNA clones

Five cDNA clones were isolated from T47D breast cancer cells cDNA library (T) and 15 cDNA clones were isolated from placenta cDNA library (P). The two clones with the largest inserts (1 KB) were T16 from T47D breast cancer and p4, 7 from placenta.

The sequence of the full length cDNA (0.9 KB) from clone T16 revealed a sequence of 890 bp; 109 bp in the 5' UTR 495 bp (165 aa) in the coding region and 286 bp in the 3' UTR. Full sequences are shown in Fig. 1.

The nucleic acid sequence of cDNA from clone p4, 7 revealed a sequence of 890 bp which is completely homologous to the known FTH sequence (as compared in Fig. 2) and represents a normal ferritin heavy chain (Cohen *et al.*, 1996, Drysdale 1988). Partial homology was found between clone p4, 7 and clone T16 (Fig. 2A, 2B). The homologous sequences were clone p4, 7 139-511 and clone T16 87-460. The later

included 22 bp in the 5' UTR followed by 351 bp (117 aa) in the above coding region. No further homology was found between the above two clones. As can be seen in Fig. 2A and 2B, homology is indicated by a broken line.

5

Example 6 Comparison to Gene Bank data

Comparison of the new sequence of T16 to sequences in the Gene Bank and EMBL database show only a segment from 463-671 bp matching a segment of human mitochondria cytochrome oxidase I (COI) 6486-6694 bp (Fig. 3). However, the predicted 48 amino acids of T16 did not match the CO I sequence. There was no further homology found in the Gene Bank for T16; 671-890 bp or I-87 bp.

Example 7 PCR amplification of a T16 compatible placental cDNA

A placenta λ gt11 cDNA library was used to amplify by PCR a T16 compatible cDNA from placenta. This was performed using the T16 specific primers i.e. λ gt11 F (1060) or λ gt11 R (1061) and primer 16R for the 5' end and primer SPF for the 3' end (Table 1; Fig. 7) schematically as shown below:

20

1060 5' _____ 16 3'

5' SPF _____ 1061 3'

25

The PCR products obtained from the placenta cDNA library using the above T16 (presented in Fig. 4). There was only a small difference in the 5' non coding region which included 2 single nucleotide substitutions, as well as a single base insertion and one deletion (Fig. 4).

Verification of the sequence was carried out on cDNA obtained from RNA by reverse transcriptase and PCR amplification (RT-PCR). The RNA was isolated from human T47D breast cancer and HBL 100 breast epithelial cell lines, and from human peripheral blood lymphocytes (PBL), non
 5 activated and from concanavalin activated PBL.

RNA (5 µg) from the different cells was used to prepare cDNA by reverse transcriptase using random primers. T16 cDNA as amplified by PCR using the following primers:

10 5' BNCF and 3' 16R (Table 1; Fig. 7), followed by nesting with the primers 5' BNCF-3' 17R and 5' 17F-3' 16R (Table 1; Fig. 7).

5' BNCF _____ 16R 3' 1st PCR

15 5' BNCF ----- 17R 3' nested PCR

5' 17F _____ 16R 3' nested PCR

Nesting was necessary since the first PCR amplification did not yield a visible PCR product.

20 The sequences obtained from the isolated cDNA products from the above cell sources revealed that all of them including T47D breast cancer had a sequence identical with the sequence obtained from the T16 homologous cDNA from placenta (Fig. 4), i.e. it included the substitution insertion and deletion in the non-coding region.

25 These results suggest that the sequence differences in the T16 cDNA clone isolated from the T47D cDNA library were a mistake occurring in the formation of the library. The final nucleotide sequence of T16 and the deduced amino-acid sequence are presented in Fig. 5.

Example 8 Expression of T16 in various tissues

The expression of T16 gene in a variety of human tissues, including breast epithelial and breast cancer cell lines were analyzed by northern blotting, according to state of the art procedure and the results are shown in Fig. 6A and 6B.

The northern blot revealed a 0.9 kb transcript in all the cells tested.

However, as seen in Fig. 6A, 6B, the relative expression of T16 mRNA and FTH revealed that there was over-expression of T16 mRNA only in breast cancer cells indicating that the T16 can be used as a marker for breast cancer.

The relative amount of each tissue mRNA indicates that all the peaks on the blot for a single probe FTH (normal H chain) or T16 (OFF1) amount to 100% and therefore each mRNA bar represents a ratio relative to the other tissues. In order to ensure that the amount of RNA loaded into the gel is equal, a household gene i.e. actin was probed. The numbers are corrected according to ratio of actin. On the same blot therefore if both mRNA (FTH and T16 (OFF1)) are expressed similarly (if one is low/or high the other is also low/or high) then there is no difference in expression between the two mRNAs. However, only in breast cancer there is evidence that T16 (OFF1) is high as compared with FTH but not in HBL cells which is a cell line derived from normal lactating breast. These results clearly indicate that the method of detecting OFF1 is valid for differentiating between breast cancer and cells derived from normal lactating breast.

Example 9 Preparation of OFF1 - Fusion Protein

Materials and Methods

Construction of *E. coli* Strains Expressing Glutathione S-Transferase (GST) and OFF1 Fusion Proteins

The expression vector (pGEX-5X-1) used for gene fusion construction was the GST Gene Fusion System (Pharmacia). The OFF1 coding region (designated as "FL", full-length) of about 0.5 kb was prepared by PCR with the following 5' end primer:

10 5' GTGGGATCCCCATGACGACGCGTCCA, in order to add a Bam HI
BamHI

site 1 base upstream from the start codon ATG and with the 3' end primer

15 5' CCGG CTCGAG TCA GGG TGA CCG AAA AAT CAG 3' in order to
XhoI
add an XhoI site after the stop codon TAA using the PCR kit (Perkin-Elmer/Cetus).

A deletion construct for encoding the unique C-terminal 48 aa, designated as "C48", of the OFF1 was also prepared from "FL" OFF1 PCR product, by cleavage with restriction enzymes 5' ECORI and 3' XhoI. The PCR program was as follows (cycles):

1. 94° - 2 min.
2. 94° - 1 min.
- 25 3. 50° - 2 min.
4. 72° - 3 min.
5. followed by 28 times
6. 94° - 1 min.
7. 50° - 2 min.
- 30 8. 72° - 10 min.

The PCR products were gel-purified and then ligated into the pGEX 5X-1 plasmid at 5' BamHI and 3' XhoI sites. The resultant recombinant:

plasmids can produce fusion proteins in which the N-terminus is the GST and the C-terminus is the OFF1, or c48 of OFF1. *E.coli* strain BL-21 was transformed with the vector alone or the two recombinant plasmid DNA to produce pGST cells, pGST-FL cells, pGST-C48 cells following the standard protocol (Maniatis Sambrook J. Fritsch EF & Maniatis T., Molecular Cloning: A Laboratory Manual (1989) (Cold Spring Harbor Lab. Press Plainview, N.Y. 2nd Ed.)).

Growth of *E.coli* Cells and Expression of OFF1 Fusion Protein in *E.coli*

10 Wild-type and transformed *E.coli* cells were grown in Luria-Bertani (LB) broth containing 100 µg/ml of ampicillin at 37°C overnight. The overnight cultures were diluted 1000-fold using fresh LB broth plus ampicillin, and incubation continued at 37°C. For growth curve determination, samples were taken every 30 mins. to measure the optical
15 density at 600 nm. To test the induction conditions for fusion protein expression, the diluted overnight cultures were grown at 37°C until mid-long phase (3-4 h, or $OD_{600} \approx 0.6$). Isopropyl β-D-thiogalactopyranoside (IPTG) was then added to a final concentration of 1 mM, and incubation was continued at 37°C for 4 hours. After IPTG induction, the cultures were
20 harvested and cell pellets were obtained by centrifugation. Pellets were resuspended in 100 µl of Laemmli sample buffer. Thirty micrograms of protein samples, determined by the Bradford assay, was subjected to SDS/PAGE.

25 SDS/PAGE and Western Blot Analysis

One-dimensional SDS/PAGE was performed according to Laemmli using 12.5% (wt/vol) polyacrylamide gels. For immunoblotting, proteins were transferred from polyacrylamide gels to immobilon polyvinylidene difluoride membranes (Millipore) with Tris/glycine electroblotting buffer

according to Towbin *et al.*⁽²⁹⁾. Protein bands cross-reacting with CM-H-9 monoclonal antibody (MoAb) were identified by reaction with horseradish peroxidase conjugated with goat anti-mouse IgG (Bio-Rad). The conditions of immunoreactions were according to the manufacturer's specification (DAKO).

Method for preparation of OFF1 recombinant protein

Day 1:

- Inoculate a single colony of transformed bacteria into 50 ml LB-Borth (with the appropriate antibiotics) and grow overnight.

Day 2:

- Add the 50 ml culture to 1000 ml LB-Borth (with the appropriate antibiotics) and grow for 2 hrs at 37°C (around 0.8 O.D.).
- Add this culture 100 µl of IPTG (stock solution of 1M) and continue growth for 4 hrs at 37°C for 5 hrs at 30°C.
- Centrifuge for 20 mins. at 4°C, 4000 rpm with rotor GS-3.
- Discard the supernatant and suspend in 20 ml ice cold PBS+0.1% Triton-X100.
- Sonicate 90/7 for 10 sec, three times on ice (in 50 ml tube)
- Spin 2300 rpm at 4°C for 10 min.
- Collect supernatant in 50 ml Falcon tube
- Add 1 ml of Glutathion-Sepharose 4B Beads (stock 50% beads in PBS).
- Incubate for 5-30 min. at RT or overnight at 4°C with rolling.
- Collect the beads by centrifuging 1 min, 2000 rpm.
- Wash the beads 3 times with ice cold PBS.
- Elute protein with 1 ml of 50 mM Tris-HCl pH 8 containing 15 mM Glutathione and 10 mM DTT.

- Dialyze (twice) against PBS containing 30% Glycerol.
- Store aliquots in -20°C.

The results are shown in Fig. 9A and 9B and clearly indicate (also with verification of binding to specific monoclonal antibodies) that recombinant protein was expressed in the host cell.

Example 10 Expression of T16 gene as a biomarker in breast cancer

The presence and over expression of T16 transcript in breast carcinoma is consistent with the differential cDNA cloning strategy which suggest its utility as a biomarker in breast cancer detection.

- 25 patients suspected of having cancer were tested for their T16 transcript by using RT-PCR in their peripheral blood as described in the experiment.

All 25 patients underwent biopsy and their condition assessed independently by the pathology department of Rabin Medical Center, Israel.

- The results are shown in Table 2, wherein (-) = no product, (\pm) = very faint band, (+) or (++) = strong bands.

- As can be seen, of the 13 cancer patients 12 were identified by over expression of T16 with a single false-negative result (Case 12) which was clinically assessed as having cancer and not diagnosed by T16 over expression. These results indicate a false-negative level of about 9%. Of the 15 patients positively identified by the T16 transcript, 3 were not clinically diagnosed as having cancer, i.e. three false- positive results, indicating a 20% level of false-positive results.

Table 2

PCR Amplification of T16 transcripts in blood of patients
with suspected breast cancer

No. **	RTPCR 17-SPR*	Diagnosis of breast biopsy	Remarks
1	++	Inf. Duct Ca T 1.6 No/12	Cancer
2	-	Fibrocystic Disease	
3	-	Fibrocystic Disease	
4	+	Inf. Duct Ca	Cancer
5	-	Fibrocystic Disease	
6	-	Fibrocystic Disease	
7	++	Inf. Duct Ca GII T1.4 No/11	Cancer
8	++	Lobular Ca In-Situ	
9	-	Fibrocystic Disease	
10	-	Atypical hyperplasia	
11	+	Inf. Duct Ca GIII T1,2 N?	Cancer
12	-	Inf. Duct Ca GII T2 No/8	Cancer
13	+	Atypical hyperplasia + Fibrocystic Disease	
14	+	Inf. bular Ca. T1.8 N1/10	Cancer
15	+-	Large lactiferous ducts. Periductal lymphoid Inf.	
16	++	Inf. Duct Ca T2 No/15	Cancer
17	+	Inf. Duct Ca T2 No/8	Cancer
18	+	Fibrocystic Disease	
19	++	Inf. Duct Ca GII T2.5 N/9	Cancer
20	+	Inf. Duct Ca GIII T1 No/8	Cancer
21	-	Fibrocystic Disease Ductectasia	
22	+	Introducal papillary Ca T0.5	
23	++	Inf. Duct Ca GII T1 No/15	Cancer
24	-	Fibrocystic prol. Ductectasia Atypical hyperplasia	
24	+	Inf. Duct Ca GII-III T2 No/13	Cancer

5

- * Amplification of T16 mRNA transcripts in blood of patients prior to breast biopsy
 (-) No PCR product (+/-) Very faint PCR product (+), (++) Positive PCR product

10

Example 11 The effect of OFF1 on granulocyte-monocyte propagation cells

OFF1 was purified from term placenta as previously described in U.S. 4,882,270 and U.S. 4,954,434 and will be termed hereinafter also as p43.

Mouse MoAb CM-H-9 was produced against human placental ferritin as previously described in the above two U.S. patents. The MoAb was obtained from ascites fluid following precipitation with 50% saturated ammonium sulphate solution, and purification on sephadex G-200 column.

CFU-GM ASSAYS

Bone marrow samples from 11 healthy volunteer donors were processed by density gradient separation using Histopaque-107 (Sigma diagnostics, St. Louis, MO, USA) to obtain a purified population of mononuclear cells. Colony assays were performed in a plating medium containing final concentrations of 0.92% methyl cellulose (M-281 powder, 4,000 centipoise, Fisher Scientific Co., Fair Lawn, NJ, USA), rehydrated in Iscove's modified Dulbecco's medium containing 36 mM sodium bicarbonate (Gibco, Grand Island, NY, USA), 30% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) 0.292 mg/ml glutamine, 100 U/ml penicillin and 0.01 mg/ml streptomycin (Biological Industries, Beit Haemek, Israel).

Growth factors used were 15-30 mg/ml GM-CSF Leucomax (Sandoz Pharma) and 5% vol/vol human phytohemagglutinin-M (Difco Laboratories, Detroit, MI, USA)-induced conditioned medium (Cond. Med.) p43 (PLF) was added at concentration of 1 µg/mL and in neutralization experiments, p43 (PLF) was preincubated with 10x excess of CM-H-9 MoAb at 37°C for 30 min and the complex added to the assay as above.

The colony assay medium contained 10^5 mononuclear cells/ml and each 1 ml was plated into triplicate wells (333 μ l/well) of a 24 well tissue culture plate (Greiner, Germany). Water was added to spaces between cells to maximize humidity during incubation of the cultures. The cultures were incubated at 37°C in 5% CO₂ and 55% relative humidity. Plates were scored after 14 days for colonies containing more than 50 cells.

RESULTS

Effect of OFF1 protein (p43 (PLF)) on human CFU-GM growth

10 in-vitro: Dose response

(p43 (PLF)) was tested for its capacity to influence colony formation of human bone marrow progenitor CFU-GM. p43 alone exhibited a concentration dependent stimulatory effect on bone marrow progenitor cells obtained from three donors. The highest number of colonies was obtained with 1 μ g/1mL of p43 (Fig. 10). At higher concentrations the number of cells was lower. All subsequent experiments were further carried out at concentration of 1 μ g/mL of p43 (PLF).

Comparison of the stimulatory effect of p43 (PLF) and GM-CSF

20 The mean number of colonies obtained following treatment of bone marrow cells from 11 donors with p43 (PLF) was 169 +/- 216 significantly higher ($p=0.028$) than in medium only (18 +/- 21) (Table 3). The stimulatory effect of p43 (PLF) was abolished following preincubation with its specific MoAb CM-H9 Mean CFU-GM - 7 +/- 10) (Fig. 11). The number of colonies obtained with p43 (PLF) was not significantly different than the number obtained with Cond. Med. (227 +/- 223) or with GM-CSF (276 +/- 257) (Table 3). When the treatment combined both GM-CSF (15 μ g/mL) and p43 (PLF) the mean number of colonies obtained with the

mixture increased to 353 \pm 236 colonies, but it did not reach a statistical significance compared to each factor alone.

It is concluded that p43 (PLF) known to act as an immunosuppressive cytokine is active as a growth factor on human bone marrow progenitor cells.

Table 3

The effect of p43 (PLF) on the generation of CFU-GM formation by normal human bone marrow.
Comparison with conditioned medium and with GMCSF.

Bone Marrow No.	None	T R E A T M E N T		
		Cond.Med.	p43 (PLF) (1 μ g/ml)	GM-CSF (15ng/ml)
1	8	522	280	144
2	46	758	748	396
3	46	322	152	114
4	2	90	28	66
5	2	134	94	136
6	8	170	6	250
7	0	52	0	50
8	4	102	242	74
9	0	54	8	520
10	34	170	210	884
11	48	126	94	405
AVG.	18	227	169	276.2
STD.	21	223	216	257
MEDIAN	8	134	94	144
RANGE	0-48	52-758	0-748	50-884

Example 12 Effect of C48/OFF1 on induction of TH1/TH2 cytokine secretion

Studies of animal models of organ-specific autoimmune diseases suggest that a cascade of autoreactive T helper 1 (Th1)-type inflammatory responses mediates the disease process. Although the initial autoimmune response is limited in its recognition of self-antigens, it subsequently expands to react with additional target tissue antigens.

Based on the antagonistic functions of different T-cell subsets, a paradigm became popular which held that induced regulatory responses (such as Th2 cells) could be used to downregulate proinflammatory pathogenic autoimmune responses and inhibit disease progression. A polypeptide encoded by the DNA molecule of the invention may be used for this purpose.

Methods

In-vitro activation of human lymphocytes without and with C48/OFF1 treatment was carried out in a mixed lymphocyte culture (MLC). Human peripheral blood lymphocytes (2×10^6 /ml) (responder cells) were incubated with non-related Mitomycin C ($40 \mu\text{g}/\text{ml}$) treated lymphocytes (2×10^6 /ml) in RPMI-1640 medium containing 10% Fetal Calf serum and antibiotics. The cultures were incubated for 24h in a humidified incubator at 37°C with 5% CO_2 . Supernatants were collected and the concentration of the secreted cytokines were measured using an ELISA assay kit. The level of IL-2 R, and interferon (Th1 cytokines) and IL-6 and IL-10 (TH2 cytokines) were determined.

As may be seen in Table 1, activation of lymphocytes following treatment with C48 (OFF1) induced the secretion of exceedingly high levels of IL-6 and IL-10 (TH2-type), which are known to inhibit type 1 immune responses (IL-2 and Interferon γ) as indicated in Fig. 12.

Table 4

Effect of C48/OFF1 on cytokine secretion by activated human lymphocytes in MLR in-vitro.

Cytokine (u/mL)	(1) Treatment of MLR	
	None	C48/OFF1
sIL-2R	50	50
γ Interferon	53	46
IL-6	20,580 +/- 1892	170,779 +/- 58,800
IL-10	54 +/- 23	1125 +/- 332

- 5 sIL-2R = Soluble interleukin 2 receptor

It is therefore concluded that C48/OFF1 induces preferentially a TH2 type immunoresponse associated with diminished cellular immunity.

Example 13 Effect of C48 (OFF1) on Zymosan induced arthritis (ZIA)

10 in mice

Materials and methods

Animals: Female ICR mice were obtained from our institution's breeding facilities and were fed a standard diet and tap water *ad libidum*.

- Induction of zymosan-induced arthritis: A homogeneous suspension of 25
 15 mg zymosan A (*Saccharomyces cerevisiae*), dissolves in 1 ml
 endotoxin-free saline. was obtained by boiling twice followed by sonic
 emulsification. Arthritis was induced by intrarticular injection of 0.5 mgr/20
 1 zymosan into each right and left knees.

- Assesment of ZIA: Mice were evaluated at 14 days post zymosan
 20 injections: Swelling was quantitated by measuring the thickness of the

knees with a caliper. at 14th day post ZIA and compared to the thickness prior to ZIA.

Histologic processing and analysis of knee joints: Knee joints were dissected, fixed, decalcified, dehydrated, and embedded in paraffin.

5 Standard frontal section of 7 μ m were prepared and stained with eosin-hematoxylin.

Disease severity was assessed by calculation of arthritic index as follows:

Semiquantitative assessment of pathologic findings was performed using a grading system, consisting of five grades. **Grade 0:** normal synovia; **grade 1:** 1-5 cellular infiltrates in the microscopic field;
10 **grade 2:** 6-20 cellular infiltrates; **grade 3:** 21-50 infiltrates; **grade 4:** more than 56 cells.

Lymphocytic nodules: **grade 1:** 1-3 nodules per section; **grade 2:** 4-10 nodules; **grade 3:** 11-20 nodules; **grade 4:** more than 20
15 nodules.

Histiocytes grading: **grade 1:** 1-10 cell; **grade 2:** 11-20 cells; **grade 3:** 21-30 cells; **grade 4:** more than 30 cells.

Synovial thickness: **grade 0:** presence of 1 layer of synovial cells; **grade 2:** 2-3 layers; **grade 3:** 4-6 layers; **grade 4:** more than 6
20 layers.

The presence of pannus was considered evidence of severe synovitis.

Treatment with C48 (OFF1): Three groups of 10 mice each, were treated by daily intraperitoneal injections of either PBS (control group) or C48 (30 μ g/per mouse).

25 Statistical analysis: The significance of comparisons between means was assessed by Student's t-test.

Results

Effect of C48 administration on ZIA: Two group of mice (n=10 each) were treated by daily i.p. injections from day 1 up to 10 (10 days), or from day 3 to 10 (7 days) (C48 delayed) following intraarticular zymosan injections.

- 4 Groups of ZIA mice (n=10) which received daily injections of PBS served as control.

Arthritis developed in 100% of control vehicle PBS treated mice within 14 days post zymosan injections. Mean knee joint swelling of the control group was 1.24 mm whereas C48 (OFF1) treatment reduced the
10 mean swelling to 0.24 mm or to 0.33 mm in the C48 delayed treatment. (Table 5).

Table 5

Effect of C48 treatment on knee swelling of ZIA in mice

Mouse Treatment	Knee swelling (mm) Mean +/- SD (n=10)	Significance p ¹
PBS (control)	1.24 +/- 0.59	
C48	0.24 +/- 0.56	0.001
C48 delayed	0.33 +/- 0.57	0.002

15

¹ p = Significance of difference from control by student's t test.

Administration of C48 from day 1 to 10 or from day 3 to 10 significantly reduced the severity of arthritis to very mild compared with
20 ZOA mice treated with PBS alone, as indicated by the arthritic score (Table 6).

Table 6

Effect of C48 treatment on disease severity of ZIA in mice

Mouse Treatment	Arthritis Score Mean +/- SD (n=10)	Significance p ²
PBS (control)	1.7 +/- 0.78	
C48	1 +/- 0.83	0.0008
C48 delayed	0.9 +/- 0.83	0.0001

¹ Score represents the mean grades of pathological findings.

² p= Significance of difference from control by Student's t test

No significant difference was found between the groups of mice treated with C48 (OFF-1) between day 1 to 10 or only from day 3 to 10.

Example 14 Protocol for treatment with c48/OFF1 of patients with Rheumatoid arthritis

Patients: Men and women 18 years of age or older with rheumatoid arthritis according to the criteria of the American College of Rheumatology.

Treatment: Patients may be treated with c48 or OFF1 at a dose of e.g. 0.5-5mgr per kilogram weight injected subcutaneously twice weekly for three months. Other treatment protocols may be determined by those skilled in the art. Treatment may be repeated with disease recurrence.

Example 15 Therapeutic Agent In Transplantation

Successful transplantation of organs requires the use of agents capable of suppressing the immune response against alloantigens.

Use of these nonspecific immunosuppressive drugs, however, can lead to the development of opportunistic infections or secondary cancer. For this

reason. a way of specially suppressing alloreactive T cells without inhibiting the entire T cell repertoire is an important goal of transplantation immunology.

In the case of bone marrow transplantation (BMT), T cells in the donor marrow are the cause of graft-versus-host disease (GVHD). We hypothesized that the induction of anergy in donor T cells that have the potential to react against the recipient's alloantigens might ameliorate GVHD while preserving the rest of the T cell repertoire.

The present treatment protocol was undertaken to develop *ex-vivo* treatment with OFF1 to induce a state of alloantigen-specific tolerization resulting in the lack of GVHD generation *in vivo* and development of bone marrow chimerism.

Methods

15 Animals: C57BL/6 (H2^b) and BALB/c (H2^d) mice were purchased from Harlane. Donors and recipients were 8-10 wk of age at the time of BMT. All mice were housed in a specific pathogen-free facility in microisolator cages. Ex-vivo induction of anergy in mixed lymphocyte reaction (MLR): C57Bl donor bone marrow (responder) was harvested 2 days before transplantation. 20 Balb/c recipient (stimulator) spleen cells were irradiated at a midplane dose of 3300 cGy. The cells were resuspended in RPMI-1640 medium with 5% mouse serum to 4x10⁶/ml and incubated with 1 µg/ml of OFF1 for 30 min.

The erythrocyte depleted mononuclear cell fraction of the marrow was resuspended at a concentration of 4x10⁶/ml in the above medium in a Donor: 25 Recipient ratio of 1:1. The cells were cultured in tissue culture flasks for 36 hours at 37°C in 5% CO₂, washed and resuspended to 2x10⁷ cells/1ml in PBS containing OFF1 (1 µg/ml).

Bone marrow Transplantation (BMT): Balb/c recipients were sublethally irradiated by exposing mice to 5:5 GRAY total body irradiation from a ¹³⁷Cesium source at a dose rate of 85 cGY/min.

Day 2 MLR cultured cells (10⁷/0.5ml) were injected intravenously to
5 BALB/c mice pretreated with OFF1 ((10 µg) injected i.p.) (BMT + OFF1).
10 Control mice were injected with MLR bone marrow cells treated with PBS.

Mice were treated for 14 days by i.p. injection of 10 µg OFF1. Control mice were injected with PBS (BMT+PBS)

Flow cytometry: Bone marrow (B.M.) and splenocytes (spl.) were removed
10 from transplanted Balb/c mice 6 weeks post transplantation and tested with
anti-K^b monoclonal antibody for the presence of C57Bl Donor lymphocytes.

All results were obtained using FACS (Beckton Dickenson). Forward and side-scatter setting were gated to exclude debris. 10,000 cells were analyzed for each determination.

15 Immunologic assay: Splenocytes from bone marrow transplanted (BMT) Balb/c mice were reacted as above in MLR against the donor C57Bl splenocytes or against non-related splenocytes, to measure their immunoresponsiveness to the corresponding alloantigens.

20 Results

In a representative experiment, (results not shown) a population of K^b positive cells both in the bone marrow (BM) and in the spleen (Spl) was evident only in Balb/c (K^d) BMT treated with OFF1 and not in BMT treated with PBS (none). These results indicate that treatment of BMT with OFF1
25 resulted in development of hematopoietic chimerism .

Example 16 Protocol for transplantation of anergic haploidentical bone marrow in human patients

Recipients: Patients with haploidentical bone marrow to the donor (i.e., marrow from a donor who shared only one or two major histocompatibility-complex haplotypes with the recipient).

Treatment: The patients undergo leukopheresis to collect 200 million to 600 million mononuclear cells per kilogram of body weight for use as the recipient's alloantigen-presenting cells. These cells are cryopreserved with the use of a standard method. Subsequently, the patient receive 1400 cGy of total-body irradiation followed by cyclophosphamide, and methylprednisolone.

Donor marrow is harvested two days before transplantation. Prophylaxis against GVHD consists of a short-course of cyclosporine starting on the day before transplantation, either by continuous infusion at 0.1 mg per kilogram per hour or by a bolus of 1.5 mg per kilogram over a period of 2 to 3 hours every 12 hours.

Ex Vivo Induction of Anergy

Cryopreserved mononuclear cells derived from recipient's blood are thawed, washed, and irradiated at a midplane dose of 3300 cGy. The cells are resuspended at a concentration of 5×10^6 /ml in RPMI 1640 medium with 5% human AB serum. c48 or OFF1 is added at a concentration of 0.5-5 μ g per milliliter for 30 minutes before the addition of the donor marrow cells. The erythrocyte-depleted mononuclear-cell fraction of the marrow is resuspended at a concentration of 5×10^6 cells per milliliter in RPMI 1640 medium with 5 percent human AB serum and added to the mixture of recipients cells and c48 or OFF1, in a donor:recipient ratio of 1:1. The coculture is incubated in tissue-culture flasks for 36 hours at 37°C in 5 percent CO₂, washed, and then infused into recipient.

Engraftment of Anergic Haploidentical Bone Marrow

A median of 3 million CD34+ donor cells per kilogram of the recipient's weight are treated in the co-culture system, and a median of 2.2 million CD34+ cells per kilogram are infused into the recipient.

5 The transfused donor bone marrow may contain mature T cells, with
* medians of 28 million CD3+ cells per kilogram, including 14 million CD3+CD4+ cells per kilogram and 9 million CD3+CD8+ cells per kilogram, after the ex vivo treatment.

Example 17 *In-vitro* studies of immunoresponse to alloantigens

10 Splenocytes obtained from Balb/c mice (K^b) treated with BMT+PBS (control) and with BMT+OFF1 were used as responder cells (R) and were reacted with C57Bl (K^b) splenocytes as stimulator cells (S) in MLR.

As can be seen in Table 7, control mice (BMT+PBS) exhibited a high proliferation index (P.I.) in MLR at different S/R ratios. In contrast
15 splenocytes obtained from the BMT+OFF1 chimeric mice were completely anergic and did not react at any S/R ratios.

However, splenocytes from chimeric BMT+OFF1 Balb/c mice responded in MLR against splenocytes from a non related alloantigen from ICR splenocytes, similarly to the response of control BMT+PBS (Table 7).
20 The latter results indicate a specific anergy of BMT+OFF1 to the donor alloantigens but not to other non-related alloantigens.

Table 7

Immuneresponse of splenocytes from Balb/c mice transplanted with C57Bl bone marrow without and with OFF1 treatment.

Stimulator	S/R Ratio ¹	Bone marrow Recipient	
		BMT+PBS	BMT+OFF1
		P.I. ²	P.I. ²
(i)C57Bl	0	1	1
	0.2	5.3	1.3
	1	3.6	1
	4	3.1	1.1
(1)ICR	0	1	1
	0.2	2.8	1.8
	1	3.6	2.6
	4	15.2	9.6

¹S/R Ratio = Stimulator Responder ratio in MLR

²P.I. = Proliferation Index, compared with non activated cells (0)

In further experiments we tested the *in-vitro* effect of OFF1 treatment on responsiveness of splenocytes from control BMT+PBS against both donor C57Bl stimulators and against ICR stimulators.

As seen in Table 8, the hyper-reactivity of the control BMT+PBS mice against C57Bl stimulator was inhibited following in-vitro treatment with OFF1, similarly to the anergic chimeric BMT+OFF1. Moreover, the responsiveness of both control and chimeric BMT against ICR stimulators was similarly inhibited by in-vitro treatment with OFF1.

Table 2

Effect of *in-vitro* treatment with OFF1: Effect on the immunoresponse of Balb/c mice transplanted with C57Bl Bone marrow.

Stimulator	In-vitro Treatmen t	Bone marrow Recipient	
		BMT+PBS	BMT+OFF1
		P.I. ¹	P.I. ¹
C57Bl	None OFF1	5.3 1	1.3 1.3
ICR	None OFF1	15.2 3.5	9.6 4.7

¹P.I. Proliferation Index compared with non activated cells (0).

Conclusion

Donor marrow treated *ex vivo* with OFF1 to induce anergy can
10 reconstitute bone marrow with donor chimerism.

Chimeric BMT are anergic to the donor alloantigen but not to other
non-related alloantigens, indicating that the chimeric host is not completely
immunosuppressed and tolerance is specific. Hyporesponsiveness against
additional alloantigens can be further achieved by renewed *ex vivo* treatment
15 with OFF1 against an additional alloantigen in MLR.

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CLAIMS:

1. A DNA sequence coding for oncofetal ferritin 1 (OFF1) protein selected from the group consisting of:
- (i) a DNA sequence as depicted in Fig. 1;
 - 5 (ii) a DNA sequence as depicted in Fig. 4;
 - (iii) a DNA sequence which codes for the same amino acid sequences of (i) or (ii);
 - (iv) fragments of any of the sequences of (i) to (iii) that code for a physiologically active protein;
 - 10 (v) a DNA sequence that has at least 80% homology, as determined by hybridization under stringent conditions, to any one of the sequences of (i) to (iv) and code for a physiologically active protein; and
 - (vi) a DNA sequence that hybridizes to the sequences of (i) or (iv),
15 under highly stringent conditions, being hybridization to filter-bound DNA in 0.5M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, which can either be used as a probe for OFF1, or which encodes functionally equivalent gene product; and
 - 20 (vii) a DNA sequence that hybridizes to the sequences of (i) to (iv) under moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C yet which still encodes a functionally equivalent gene product.
- 25 2. An expression vector comprising the DNA sequence of Claim 1.
3. An expression vector according to Claim 2, being a plasmid.
4. A genetically engineered host cell containing the DNA sequence of Claim 1, operatively associated with a regulatory element heterologous to the

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DNA sequence which directs the expression of the DNA sequence by the host cell.

5. An amino acid sequence coded by the nucleic acid sequence of Claim 1.
- 5 6. A DNA sequence which is complementary to at least a portion of any one of the sequences of Claim 1, capable of being transcribed to mRNA which is an anti-sense to at least a portion of the mRNA transcribed by any one of the sequences of Claim 1, said portion being sufficient to inhibit translation of the mRNA to protein.
- 10 7. An anti-sense mRNA sequence transcribed from the DNA of Claim 6.
8. A pharmaceutical composition comprising the expression vector of Claim 3.
9. A pharmaceutical composition comprising the amino acid sequence
15 of Claim 5.
10. A pharmaceutical composition according to Claims 8 or 9, for immunization against cancer.
11. A pharmaceutical composition according to Claim 10, for immunization against breast cancer.
- 20 12. A pharmaceutical composition according to Claims 8 or 9, for the treatment of transplant rejections, autoimmune diseases, pathological pregnancies and for enhancing fertilization rates during IVF treatment.
13. A pharmaceutical composition according to Claims 8 or 9 for use as a growth factor of bone-marrow progenitor cells.
- 25 14. A pharmaceutical composition according to Claim 13, wherein the cells are granulocyte monocytes.
15. A growth factor for bone marrow progenitor cells comprising as an active ingredient the amino acid sequence of Claim 5.
16. An expression vector comprising the DNA of Claim 6.

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17. A pharmaceutical composition comprising the expression vector of Claim 16.
18. A pharmaceutical composition comprising the anti-sense mRNA sequence of Claim 6.
- 5 19. A pharmaceutical composition according to Claim 17 or 18, for the treatment of cancer.
20. A pharmaceutical composition according to Claim 19 for the treatment of breast cancer.
21. A pharmaceutical composition according to Claim 17 or 18, for the
10 induction of abortion.
22. A method for the diagnosis of cancer comprising: detecting elevated to levels of mRNA transcribed from DNA sequences depicted in Fig. 1 or Fig. 4.
23. A method according to Claim 22, wherein the cancer is selected from
15 the group consisting of: breast cancer, hepatoblastoma, leukemia, Hodgkin's and non-Hodgkin's lymphomas and embryonal tumors.
24. A method for the detection of Down's Syndrome, comprising detecting elevated levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.
- 20 25. A method for the detection of pathological pregnancies comprising detecting decreased levels of mRNA transcribed from the DNA sequence of Fig. 1 or 4.
26. A method according to Claim 25, wherein the pathological pregnancy is selected from the group consisting of: spontaneous abortion and
25 miscarriage, premature contractions, toxemia, premature delivery.
27. A method according to any one of Claims 22 to 26, wherein the level of the DNA expression is detected using RT-PCR.
28. A method for isolating the DNA sequence of Fig. 1 or 4, substantially as hereinbefore described.

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5'	TTGACACCAG	ACCAACTGGT	AATGGTAGCC	ACCGCGGCTC	AGCTGGGATT	CCTAAAAATG
TAATGCACAC	TCCATTGGCAT	TCAGCCCGCC	TCTCCTTACT	CGCCCGC <u>ATG</u>	ACGACCGCGT	
CCACCTCGCA	GGTGGCCGAG	A ^u ACTACCA ^u CC	AGGACTCAGA	GGCCGCCATC	AACCGCCAGA	
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCCGG	
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAATCTCAT	GAGGAGAGGG	
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCTTCAGG	
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGGCT	GAATGCAATG	GAGTGTGCAT	
TACATTTGGA	AAAAAATGTG	AATCAGTCAC	TACTGGAATT	CCCTTCTCCT	ATCTCTCCCA	
GTCTTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC	
GACCCCGCGG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC	
CC <u>TGA</u> AGTTT	ATAITCTTAT	CCTACCAGGC	TTGGGAATAA	TCTCCCATAT	TGTAACCTAC	
TACTCCGGAA	ATCGCTGTCT	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC	
CTAATTGGAA	GCGCCACCCCT	AGCAATATCA	ACCATTAAAC	TTCCCTCTAC	ACTTATCATC	
TTCACAATTC	TAATTCTACT	GACTATCCTA	GAAATCGCTG	TGCGCTTAAT	CCAAGCCTAC	
GTTTTACAC	TTCTAGTAA	GCCTCTACCT	GCAGACAAC	ACATAAAAAA	AA	3'

Fig. 1

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Fig. 2A

CLONE p47

GGGGGACGGAAACCCGG

CGCTCGTTCCCCAGCCCGCGCCGCCCATAGCCAGCCCTCCGTAC

CLONE T 16TTGACACCG

CTCTTACCGCACCCCTCGGACTGCCCAAGGCCCGCGCCGCTCC
ACACCAACTGGTAATGGTAGCGACCGCGCTCAGCTGGAATTCACAAA

AGCGCGCGCAGCCACCGCGCGCGCGCCCTCTCCTTAGTCGCCGCC
AATGTAATGCACACTCCATTGCATTAGCCCGCCTCTCCTTAGTCGCCGCC

ATG	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG
ATG	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC
TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT
TTT	CTT	CAC	CAA	TCT	CAT	GAG	GAG	AGG	GAA	CAT
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT
GTG	AAT	CAG	TCA	CTA	CTG	GAA	CTG	CAC	AAA	CTG
GTG	AAT	CAG	TCA	CTA	CTG	GAA	<u>TTC</u>	<u>CCT</u>	<u>TCT</u>	<u>CCT</u>
GCC	ACT	GAC	AAA	AAT	GAC	CCC	CAT	TTG	TGT	GAC
ATC	TCT	CCC	AGT	CCT	AGC	TGC	TGG	CAT	CAC	TAT

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TTC	ATT	GAG	ACA	CAT	TAC	CTG	AAT	GAG	CAG	GTG
ACT	ACT	AAC	AGA	CCG	CAA	CCT	CAA	CAC	CAC	CTT

AAA	GCC	ATC	AAA	GAA	TTG	GGT	GAC	CAC	GTG	ACC
CTT	CGA	CCC	CGC	CGG	AGG	AAG	AGA	CCC	CAT	TCT

AAC	TTG	CGC	AAG	ATG	GGA	GCG	CCC	GAA	TCT	GGC
ATA	CCA	ACA	CCT	ATT	CTG	ATT	TTT	CGG	TCA	CCC

TTG	GCG	GAA	TAT	CTC	TTT	GAC	AAG	CAC	ACC	CTG
TGA	AGTTTATATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATT									

GGA	GAC	AGT	GAT	AAT	GAA	AGC	TAA	GCCTCGGGCTAATT		
GTAACCTTACTACTCCGGAAATCGCTGTCGCCTAACCGCTAACATTACTGC										

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AGGCCACCTACTCATGCACCTAATTGGAAGCGCCACCCTAGCAATATCA										

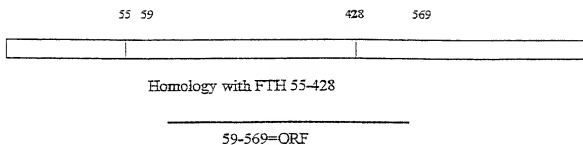
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ACCATTAACTTCCTCTACACTTATCATCTTCACAATTCTAATTCTACTG										

TTAAGTTCTTTGATTTGTACCATTCCTTCAAATAAAGAAATTTGGTACCCA										
ACTATCCTAGAAATCGCTGTCGCCTTAATCCAAGCCTACGTTTTCACACT										

AAAAA										
TCTAGTAAGCCTCTACCTGCACGACAAAACATAAAAA										

Fig. 2A Cont.

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**Fig. 2B**

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463 CTTCTCCTATCTCTCCAGTCCTAGCTGCTGGCATCACTATACTACTAAC 512
 6486 CTTCTCCTATCTCTCCAGTCCTAGCTGCTGGCATCACTATACTACTAAC 6535

 513 AGACCGCAACCTCAACACCACCTTCTTCGACCCCGCCGGAGGAAGAGACC 562
 6536 AGACCGCAACCTCAACACCACCTTCTTCGACCCCGCCGGAGGAAGAGACC 6505

 563 CCATTCTATACCAACACCTATTCTGATTTTTCGGTCACCCCTGAAGTTTAT 612
 6506 CCATTCTATACCAACACCTATTCTGATTTTTCGGTCACCCCTGAAGTTTAT 6635

 613 ATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACCTTACTA 662
 6636 ATTCTTATCCTACCAGGCTTCGGAATAATCTCCCATATTGTAACCTTACTA 6685

 663 CTCCGAAA 671
 6686 CTCCGAAA 6694

Fig. 3

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TTGACACCAG	ACCAACTGGT	AATGGTAGCG	ACCGCGCGTC	AGCTGGGATG	AAAAAATG
TAATGCACAC	TCCATTGCAT	TCAGCCCGCC	TCTCCTTAGT	CGCCGCGATG	CGTAAAATGT
AATGCACACT	CCAATTGGCAT				ACGACCGCGT
CCACCTCGCA	GGTGGCCAG	AACTACCAC	AGGACTCAGA	GGCGCCCATC	AACCGCCAGA
TCAAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	TTTGACCGCG
ATGATGTGGC	TTTGAAGAAC	TTTGCCAAAT	ACTTTCTTCA	CCAACTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCTTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAGCGGCT	GAATGCAATG	GAGTGTGCAT
TACATTGGGA	AAAAAATGTG	AATCAGTCAC	TACTGGAATT	CCCTTCTCCT	ATCTCTCCCA
GTCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC
GACCCGCGG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
CGTGAAGTTT	ATATTCTTAT	CCTACCAGGC	TTGGGAATAA	TCTCCCATAT	TGTAACCTAC
TACTCCGGA	ATCGTGTGCG	CCTAACCGCT	AACATTACTG	CAGGCCACCT	ACTCATGCAC
CTAATTGGAA	GCGCCACCGT	AGCAATATCA	ACCATTAAAC	TTCCCTCTAC	ACTTATCATC
TTCAAAITC	TAAITCTACT	GACTATCCTA	GAAATCGCTG	TCGCTTAAAT	CCAAGCCTAC
GTTTTCACAC	TTCTAGTAA	GCCTCTACCT	GCACGACAAC	ACATAAAAAA	AA

Fig. 4

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TTGACACCAGACCACTGGTAATGGTAGCGACCGCGCTCAGCTGGAATCCAAAAAATGTAATGCACACTCCATTGCATTGAGCCCGCTCTCTCTTAGTCGCCGCC

met	thr	thr	ala	ser	thr	ser	gln	val	arg	gln
<u>ATG</u>	ACG	ACC	GCG	TCC	ACC	TCG	CAG	GTG	CGC	CAG

asn	tyr	his	gln	asp	ser	glu	ala	ala	ile	asn
AAC	TAC	CAC	CAG	GAC	TCA	GAG	GCC	GCC	ATC	AAC

arg	gln	ile	asn	leu	glu	leu	tyr	ala	ser	tyr
CGC	CAG	ATC	AAC	CTG	GAG	CTC	TAC	GCC	TCC	TAC

val	tyr	leu	ser	met	ser	tyr	tyr	phe	asp	arg
GTT	TAC	CTG	TCC	ATG	TCT	TAC	TAC	TTT	GAC	CGC

asp	asp	val	ala	leu	lys	asn	phe	ala	lys	tyr
GAT	GAT	GTG	GCT	TTG	AAG	AAC	TTT	GCC	AAA	TAC

phe	leu	his	gln	ser	his	glu	glu	arg	gln	his
TTT	CTT	CAC	CAA	TCT	CAT	GAG	CAT	AGG	GAA	CAT

ala	glu	lys	leu	met	lys	leu	gln	asn	gln	arg
GCT	GAG	AAA	CTG	ATG	AAG	CTG	CAG	AAC	CAA	CGA

gly	gly	arg	ile	phe	leu	gln	asp	ile	lys	lys
GGT	GGC	CGA	ATC	TTC	CTT	CAG	GAT	ATC	AAG	AAA

pro	asp	cys	asp	asp	trp	glu	ser	gly	leu	asn
CCA	GAC	TGT	GAT	GAC	TGG	GAG	AGC	GGG	CTG	AAT

ala	met	glu	cys	ala	leu	his	leu	glu	lys	asn
GCA	ATG	GAG	TGT	GCA	TTA	CAT	TTG	GAA	AAA	AAT

val	asn	gln	ser	leu	leu	glu	phe	pro	ser	pro
GTG	AAT	CAG	TCA	CTA	CTG	GAA	TTT	CCT	TCT	CCT

ile	ser	pro	ser	pro	ser	cys	trp	his	his	thr
ATC	TCT	CCC	AGT	CCT	AGC	TGC	TGG	CAT	CAC	TAT

thr	thr	asn	arg	pro	glu	pro	gln	his	his	leu
ACT	ACT	AAC	AGA	CCG	CAA	CCT	CAA	CAC	CAC	CTT

leu	arg	pro	arg	arg	arg	lys	arg	pro	his	ser
CTT	CGA	CCC	CGC	CGG	AGG	AAG	AGA	CCC	CAT	TCT

ile	pro	thr	pro	ile	leu	ile	phe	arg	ser	pro
ATA	CCA	ACA	CCT	ATT	CTG	ATT	TTT	CGG	TCA	CCC

TGA AGTTTATATCTTATCTCTACCAGGCTTCGGAATAATCTCCCATATTGTAACCTACTACTCCGAAATCGCTGTGCGCTAACCCTAACATTACTGCAGGCCACCTACTCATGCACCTAATTGGAAGCGCCACCCTAGCAATATCAACCATTAACTTCCCTCTACACTTATCATCTTCACAATTCTAATTCTACTGACTATCCTAGAAATCGCTGTGCGCTTAATCCAAGCCTACGTTTTACACACTTTGGTACCCAAAAAAA

Fig. 5

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	HEART	BRAIN	PLACENTA	LUNG	LIVER	SK. MUSCLE	KIDNEY	PANCREAS
FTH (530bp)/ACTIN	220	146	116	187	207	155	230	290
OFF1 (SPF-16R)/ACTIN	195	115	70	60	281	127	260	170

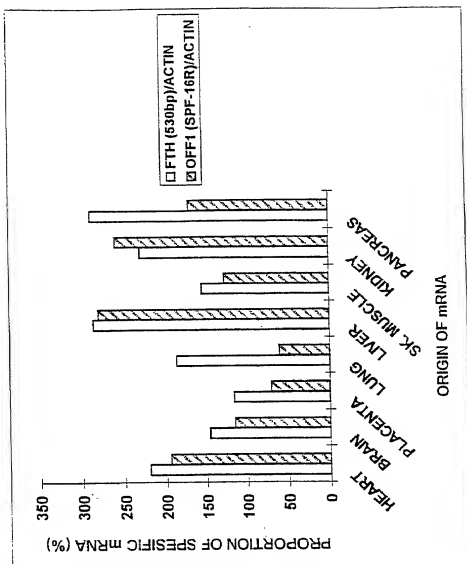


Fig. 6A

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RATIO OF ACTIN (PSL)	HBL	T47D	MCF7
FTH (530bp)/ACTIN	58.2	77.8	89
OFF1 (SPF-16R)/ACTIN	35.4	150.4	134.7

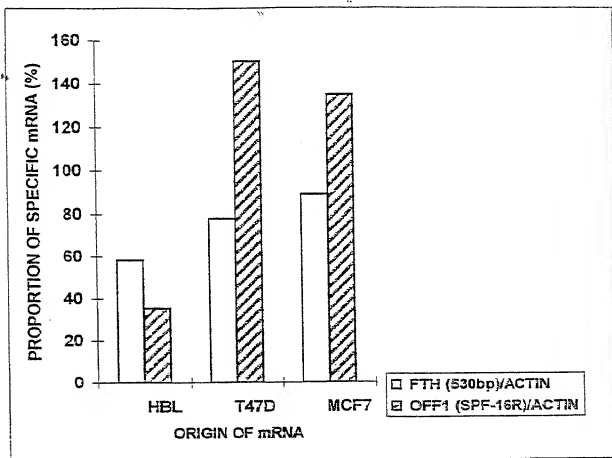


Fig. 6B

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<u>1061</u> <u>TTGACACCAG</u>	<u>ACCAACTGGT</u>	<u>AATGGTAGCG</u>	<u>BNC</u> <u>ACCGGCGCTC</u>	<u>AGCTGGAATT</u>	<u>CGAAAAAATG</u>
<u>NCS</u> <u>TAATGCACAC</u>	<u>TCCAATTGCAT</u>	TCAGCCCGCC	TCTCCTTAGT	CGCCGCGCATG	ACGACCGCGT
CCACCTCGCA	GGTGCGCCAG	AACTACCACC	<u>XI</u> <u>AGGACTCAGA</u>	<u>GGCGGCCATC</u>	AACCGCCAGA
TCAACCTGGA	GCTCTACGCC	TCCTACGTTT	ACCTGTCCAT	GTCTTACTAC	<u>17</u> <u>TTTGACCGCG</u>
<u>17</u> <u>ATGATGTGGC</u>	<u>TTTGAAGAAC</u>	TTTGCCAAAT	ACTTTCCTCA	CCAATCTCAT	GAGGAGAGGG
AACATGCTGA	GAAACTGATG	AAGCTGCAGA	ACCAACGAGG	TGGCCGAATC	TTCCCTCAGG
ATATCAAGAA	ACCAGACTGT	GATGACTGGG	AGAC <u>CGGGCT</u>	<u>21</u> <u>GAATGCAATG</u>	<u>GAGTGTGGAT</u>
TACAATTGGA	AAAAAATGTG	AATCA <u>GTCAC</u>	<u>ECOF</u> <u>FACTGGAATT</u>	<u>CCCTTCTCCT</u>	ATCTCTCCCA
GTCTAGCTG	CTGGCATCAC	TATACTACTA	ACAGACCGCA	ACCTCAACAC	CACCTTCTTC
GACCCCGCG	GAGGAAGAGA	CCCCATTCTA	TACCAACACC	TATTCTGATT	TTTCGGTCAC
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Fig. 7

WO 00/15788

PCT/IL99/00485

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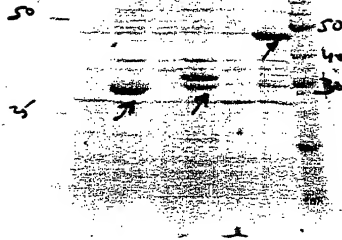
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Fig. 8

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Fig. 9

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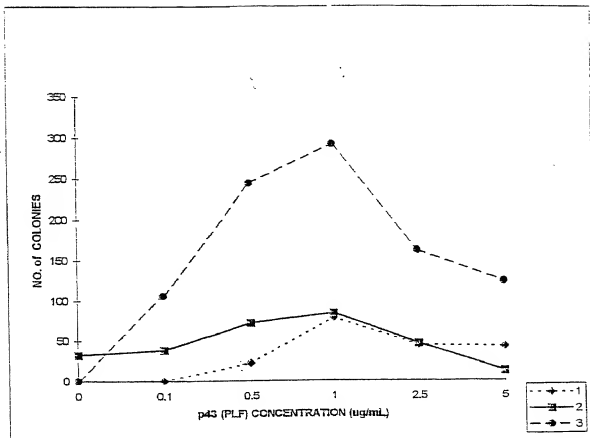


Fig. 10

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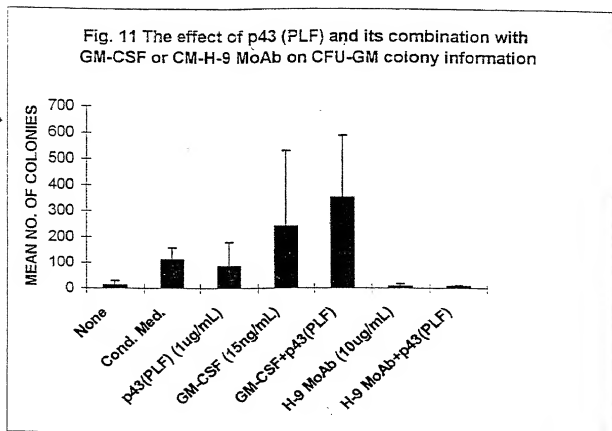


Fig. 11

15/15

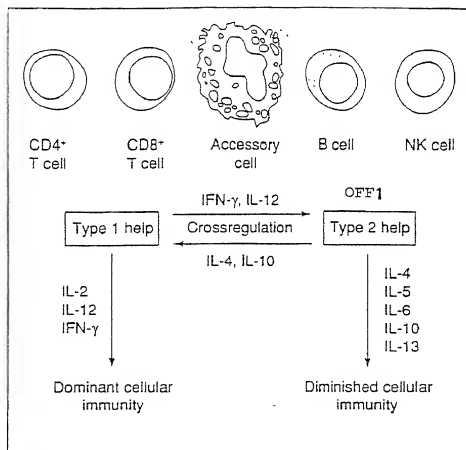


Fig. 12

Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

DNA SEQUENCE ENCODING ONOCFETAL FERRITIN PROTEIN

the specification of which (check one)

- [] is attached hereto;
[] was filed in the United States under 35 U.S.C. §111 on _____, as
U.S. Appl. No. _____*, or
[X] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international
(PCT) application, PCT/IL99/00485 _____; filed 8 September 1999 _____, entry
requested on 12 March 2001 _____; national stage application received U.S. Appl. No.
_____*; §371/§102(e) date _____ (* (* if known))

and was amended on 12 March 2001 (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 (a)-(d) and 365 (b) of any prior foreign application(s) for patent or inventor's certificate, or §365(a) of any prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked, and have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate or PCT international application having a filing date before that of the application on which priority is claimed:

126181	Israel	11 September 1998	[xx]	[]
(Number)	(Country)	(Day Month Year Filed)	YES	NO
			[]	[]
(Number)	(Country)	(Day Month Year Filed)	YES	NO

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional applications listed below:

(Application No.)	(Day Month Year Filed)
(Application No.)	(Day Month Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or under §365(c) of any prior PCT international application(s) designating the U.S., listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT international application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practitioners associated with Customer Number 001444

Direct all correspondence to the address associated with **Customer Number 001444**, which is presently:

BROWDY AND NEIMARK, P.L.L.C.
624 Ninth Street, N.W.
Washington, D.C. 20001-5303
(202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from Reinholt Cohn and Partners as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Title: DNA SEQUENCE ENCODING ONCOFETAL FERRITIN PROTEIN

U.S. Application filed _____, Serial No. _____

PCT Application filed _____, Serial No. _____

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR Chaya MOROZ		INVENTOR'S SIGNATURE <i>Chaya Moroz</i>		DATE 18.4.01
RESIDENCE Tel-Aviv, Israel <i>ILX</i>		CITIZENSHIP Israeli		
POST OFFICE ADDRESS Yehuda Hanasi 40, 69393 Tel-Aviv, Israel				
FULL NAME OF SECOND JOINT INVENTOR		INVENTOR'S SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF THIRD JOINT INVENTOR		INVENTOR'S SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
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FULL NAME OF FOURTH JOINT INVENTOR		INVENTOR'S SIGNATURE		DATE
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FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE		DATE
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POST OFFICE ADDRESS				
FULL NAME OF SEVENTH JOINT INVENTOR		INVENTOR'S SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION
NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS

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 35 40 45

Leu Lys Asn Phe Ala Lys Tyr Phe Leu His Gln Ser His Glu Glu Arg
 50 55 60
 Gln His Ala Glu Lys Leu Met Lys Leu Gln Asn Gln Arg Gly Gly Arg
 65 70 75 80
 Ile Phe Leu Gln Asp Ile Lys Lys Pro Asp Cys Asp Asp Trp Glu Ser
 85 90 95
 Gly Leu Asn Ala Met Glu Cys Ala Leu His Leu Glu Lys Asn Val Asn
 100 105 110
 Gln Ser Leu Leu Glu Phe Pro Ser Pro Ile Ser Pro Ser Pro Ser Cys
 115 120 125
 Trp His His Thr Thr Thr Asn Arg Pro Glu Pro Gln His His Leu Leu
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